

Final Report

**To the
AFIA -Liquid Feed Committee**

**Effect of Forage-Liquid Molasses Interaction on Acid-Base Physiology and
Performance in Beef Cattle**

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Brief Summary

Three treatments were utilized to investigate the effect of supplementing either molasses or molasses with a buffer on a basal forage diet with a negative dietary cation-anion difference (DCAD). The basal diet included hay, soybean meal, and Soy Chlor to elicit a negative DCAD. Treatments were: Control (-31 meq/kg DCAD), Molasses (+29 meq/kg) or Molasses+buffer (+258 meq/kg DCAD). Cow bodyweight and bodyweight change did not differ during the 42-d experiment. Likewise, cow hay dry matter intake measured by pounds or % of bodyweight was not different between treatments. Measurements of blood pH and blood gases were not affected by DCAD treatment. Urine pH was affected by the negative DCAD of the Control diet. Supplementing of Molasses or Molasses+buffer did increase urine pH relative to the Control diet. Uterine flush pH in this experiment was not affected by DCAD treatment. These results are in contrast to the findings of our previous work involving the effect of forage DCAD on performance and acid-base physiology in mature beef cows.

Rational

Our previously research (Hersom et al., 2005) reported that forage-fed beef cows with a dietary cation-anion difference (**DCAD**) of +250 mEq/kg had a 13.5% increase in dry matter intake (**DMI**) compared to forage-fed cows with a DCAD value of -9 mEq/kg. Likewise, DMI as a percent of bodyweight was decreased for cows being fed a negative DCAD diet. Decreased forage DMI would translate into decreased intake of energy, crude protein, vitamins, and minerals. Decreased nutrient intake will negatively impact cow performance, reproductive parameters, and overall profitability of the beef cattle enterprise.

In our previous work we also reported differences in uterine flush pH between cows with positive or negative DCAD values (Hersom et al., 2005). Negative DCAD forage-based diets decreased cow uterine flush pH to 6.13 which was 0.25 pH units lower than uterine flush of positive DCAD cows. Additionally, the uterine flush pH of negative DCAD cows was 0.67 pH units lower than the reported pH of bull semen. This difference in pH between the uterine environment and semen likely will have important implications for cow reproductive performance.

In our first experiment we demonstrated that negative DCAD lowers the pH of blood, urine, and the uterus environment of beef cows consuming forage-based diets. The hypothesis of the current research project was that supplementation of forage-based diets with liquid molasses and/or buffered liquid molasses may be able to alleviate the effect of negative DCAD on cow acid base physiology, urine pH, and uterine pH.

We felt that liquid molasses had the potential to be a supplement with a positive DCAD value for forage-based diets. Liquid molasses contains increased levels of both K and S (NRC, 1996). The concentration of K in molasses (4.01% DM) is an important factor in the equation for determining DCAD ($\text{mEq/kg of DM} = (\text{Na} + \text{K} + 0.15\text{Ca} +$

0.15Mg)-(Cl + 0.6S + 0.5P); Goeff and Horst, 1997). Despite the elevated level of S in molasses, the DCAD equation minimizes the contribution of S, particularly in comparison to K. Liquid molasses has an approximate DCAD value of +92 mEq/kg. The positive DCAD value of liquid molasses would provide a unique and valuable alkaline component to potentially acidogenic diets. Additionally, liquid molasses offers the advantage of being an excellent carrier for additional buffers for highly acidic forage based diets. The addition of buffering agents to liquid molasses offers an opportunity to achieve DCAD values in the positive range on highly acidic forage diets. The use of liquid molasses or molasses in combination with buffers had not been evaluated as a viable supplement to alter DCAD in forage fed beef cattle diets.

Materials and Methods

Twenty-one non-pregnant Braford cows (initial bodyweight = $1,184 \pm 90$) were utilized. Originally, 24 cows were allocated for use, however on day 0 eight cows were found to be pregnant. As a result, five new cows were placed on the experiment on d 3 of the trial. All cows were fed the same basal diet. The basal diet consisted of limpogross hay and a soybean meal/Soy Chlor supplement which supplied adequate protein and adjusted the DCAD to -59 meq/kg (Table 1). All treatments were supplemented with either corn or molasses to meet energy requirements of the cows and acted as the treatment to adjust DCAD. Three treatments were utilized; 1) **Control** basal diet with corn; 2) **Molasses** (basal diet with molasses replacing corn); 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 ft²). Limpogross hay was ground with a tub grinder to pass a 3.5 cm screen. Cows were fed their daily ration once daily for 42 day. 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 hours and shrunk bodyweight (**BW**) of the cows was taken at the initiation of the experiment and after 42 day. Cow DMI of hay was measured daily during the experiment. The daily Soy-Chlor offered in the basal diet was based upon the previous day's hay DMI. The amount of Soy-Chlor was fed to maintain a constant DCAD according to the treatment protocol despite variation in hay DMI.

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 CIDRs were removed and all cows were injected IM with 25 mg of prostaglandin F_{2α} (Lutalyse®Sterile Solution). Then on day 11 all cows were again administered 100 µg IM of GnRH.

Sample Collection and Analysis

Blood, urine, and uterine flush samples were collected from all cows approximately 2 hours after rations were offered on day 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 then 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 seconds, and then flushed out of the uterus through the catheter into a cup. Uterine flush pH was then 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

All BW, average daily gain, 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 $P < 0.05$, differences of $P < 0.10$ are discussed as potential trends.

Results

Performance

Initial and final cow BW (Table 2) exhibited more variation than in our previous DCAD experiment, as a result final cow BW did not differ between treatments ($P=0.48$). Likewise, mean ADG for the 42-day experiment was not different ($P=0.47$) between treatments. In this experiment, mean ADG was 2.22 lb/day which was considerably greater than in our previous work. Weekly hay DMI and hay DMI, % of BW did not differ between treatments (Table 3). Mean hay DMI across the six weeks of the experiment was 15.4 lb/day 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. The amount of Soy Chlor is included to demonstrate the increased amount offered as days on feed increased. The Soy Chlor offered increased as hay DMI increased to keep basal diet DCAD similar from the beginning to the end of the experiment regardless of any variation in hay DMI. We feel that this is an important improvement over our previous methodology and improves the consistency of the DCAD in this experiment. However, despite the improvement in methodology, a negative DCAD did not depress cow performance or hay DMI. Likewise supplementation using molasses with or without a buffer did not alter cow performance or intake.

The difference in hay source between our previous work (bahiagrass) and the current work (limpograss) may have marginally affected the cow performance data. More likely, the addition of corn and molasses as energy supplements in diet of the mature cows may have offset any potential detrimental effects of a negative DCAD on cow performance and intake. Mean energy supplement intake was 3.31, 3.55, and 3.97 lb/d of corn, molasses, and molasses+buffer, respectively.

Blood Acid-Base

Blood pH in on the current work (Figure 1) 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-day 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 4) 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 our previous work, however, the Low-DCAD treatment in the previous work exhibited moderate decreases in BE and bicarbonate values. In contrast, blood BE and bicarbonate concentrations of the Control diet in the current study, which had a negative DCAD value, were apparently unaffected.

Urine and Uterine

Urine pH of cows in the current study was responsive to the DCAD of the different treatments (Figure 2). 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 ($P<0.05$) 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. This difference at the initiation of the experiment was unexpected because all of the cows had been managed as group and estrus cycles synchronized prior to the initiation of the experiment. As a result of the differences in uterine pH measurement on day 0, subsequent uterine pH measurements were analyzed with day 0 as a covariate. Covariate analysis did not prove to be responsive, thus covariate analysis was not implemented for final analysis. Uterine pH of Molasses cows continued to be numerically less than Control or Molasses+Buffer cows on day 21. 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 the current study is within the range of those observed in our previous work.

Summary and Implications

The results of the current experiment are in conflict with our first study that investigated the effect of DCAD on cow performance and acid-base physiology. Cow bodyweight and intake parameters were not different as a result of treatments utilized in this experiment. It was expected that a negative DCAD of -31 meq/kg would have elicited a response in dry matter intake, blood pH, blood gases, and especially urine pH for the Control cows. The supplement ingredients (Soy Chlor, soybean meal, and corn) utilized for the Control diet were similar to our initial experiment. The basal diet had a DCAD of -56 meq/kg, the addition of the corn only moved the DCAD of the control ration to -31 meq/kg. In light of the non-responsive effect of the Control diet to elicit a response, no significant responses from Molasses and Molasses+buffer would then be expected. The results reported here are puzzling in light of the promising results of our first experiment. A factor or factors that would have exerted any effect to result in the minimal differences observed is not obvious at this time. The issue of DCAD in mixed rations and likely forage DCAD affecting animal performance and physiological mechanisms has been documented in the scientific literature. The lack of tangible results from this experiment should not diminish the potential impact that DCAD could have on beef cow performance and reproductive success. The positive role of liquid molasses was demonstrated in this experiment. The Molasses and Molasses+Buffer treatments while not significant did have blood, urine, and uterine pH values that were generally greater than the Control. The incorporation of a buffer into molasses to alleviate a negative DCAD was methodologically feasible and sound.

Literature Cited

Goff, J. P., R. L. Horst. 1997. Comparison of sulfuric acid with hydrochloric acid as a source of acidifying anions. *J. Dairy. Sci.* 75(Suppl. 1):98.

Hersom, M. J., G. Hansen, and J. D. Arthington. 2005. Effect of Cation-Anion Difference on Measures of Acid-Base Physiology in Beef Cows. pp 80-95, *Proc. 35th Liquid Feed Symp.*, Omaha, NE.

NRC. 1996. *Nutrient Requirements of Beef Cattle (7th Ed.)*. National Academy Press. Washington, DC.

Table 1. Diet composition of the rations fed to cows to result in different dietary cation-anion difference (DCAD).

Ingredient, % DM	Dietary Treatment		
	Control	Molasses	Molasses +Buffer
Low DCAD hay	76.87	75.62	73.87
Soy-Chlor	2.38	2.38	2.38
Soybean meal	3.75	4.0	4.0
Ground corn	17.0	-	-
Molasses	-	18.0	18.0
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 ^a	-31	29	258

^a DCAD = (Na + K + 0.15Ca + 0.15Mg) – (Cl + 0.60S + 0.50P).

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

Item	Dietary Treatment ^a			SEM ^b	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

^a Control, Molasses, Molasses+Buffer = -31, 29, 258 meq/kg respectively.

^b SEM = Standard error of mean, (n = 7).

Table 3. Effect of high- and low- dietary cation-anion difference (DCAD) on mature beef cow hay and Soy Chlor intake.

Item	Dietary Treatment ^a			SEM ^b	P-value
	Control	Molasses	Molasses +Buffer		
Hay DMI, lb/d					
Week 1	12.3	12.4	15.0	1.20	0.18
Week 2	16.1	15.1	16.0	0.81	0.66
Week 3	16.6	16.9	17.5	0.80	0.70
Week 4	12.0	13.1	14.4	1.09	0.28
Week 5	17.0	15.9	17.4	1.03	0.58
Week 6	16.9	15.6	17.6	1.02	0.39
Mean hay DMI, lb/d	15.0	14.9	16.3	0.84	0.39
Hay DMI, % of mean feeding BW					
Week 1	1.15	0.99	1.22	0.105	0.31
Week 2	1.51	1.21	1.29	0.089	0.06
Week 3	1.55	1.34	1.41	0.093	0.26
Week 4	1.10	1.05	1.16	0.096	0.69
Week 5	1.56	1.26	1.41	0.104	0.17
Week 6	1.55	1.24	1.42	0.103	0.13
Mean hay DMI, % of mean feeding BW	1.41	1.18	1.31	0.086	0.19
Soy Chlor offered, g/d					
Week 1	199.4	192.0	236.0	18.08	0.18
Week 2	252.4	237.1	250.6	12.60	0.64
Week 3	260.1	265.2	272.3	11.85	0.74
Week 4	191.1	206.2	225.4	17.02	0.35
Week 5	254.1	233.0	260.6	17.12	0.49
Week 6	274.5	264.3	288.8	14.73	0.48
Mean Soy Chlor offered, g/d	238.1	233.0	255.6	12.66	0.40

^a Control, Molasses, Molasses+Buffer = -31, 29, 258 meq/kg respectively.

^b SEM = Standard error of mean, (n = 7).

Table 4. Effect of high- and low- dietary cation-anion difference (DCAD) on mature beef cow blood acid-base physiology.

Item	Dietary Treatment ^a			SEM ^b	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

^a Control, Molasses, Molasses+Buffer = -31, 29, 258 meq/kg respectively.

^b SEM = Standard error of mean, (n = 7).

Figure 1. The effect of dietary cation-anion difference (DCAD) from different supplements on mature beef cow blood pH. Effect of treatment (day 0, $P=0.53$, $SEM=0.044$; day 21, $P=0.43$, $SEM=0.022$; day 42, $P=0.83$, $SEM=0.034$).

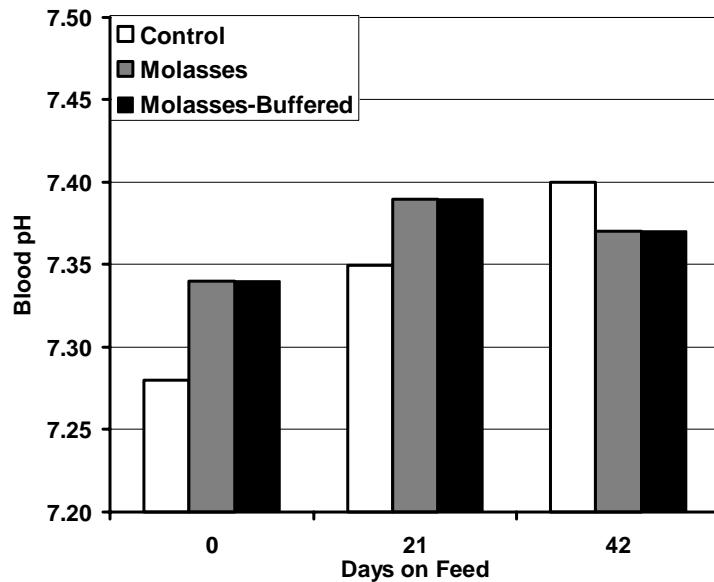
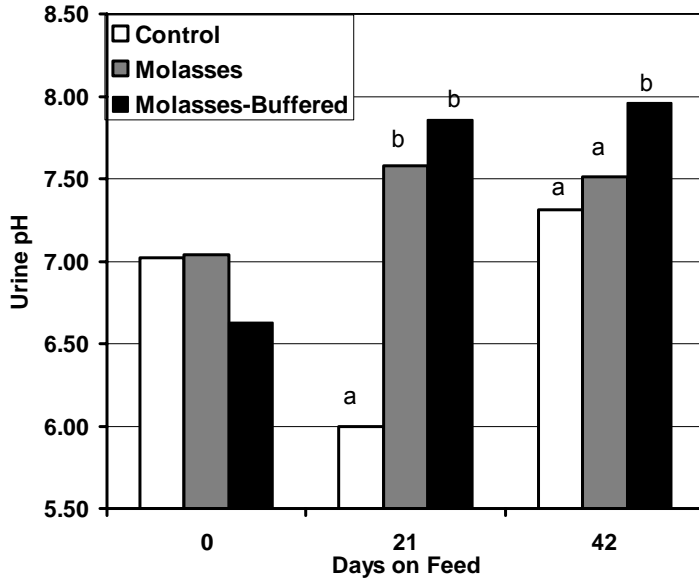
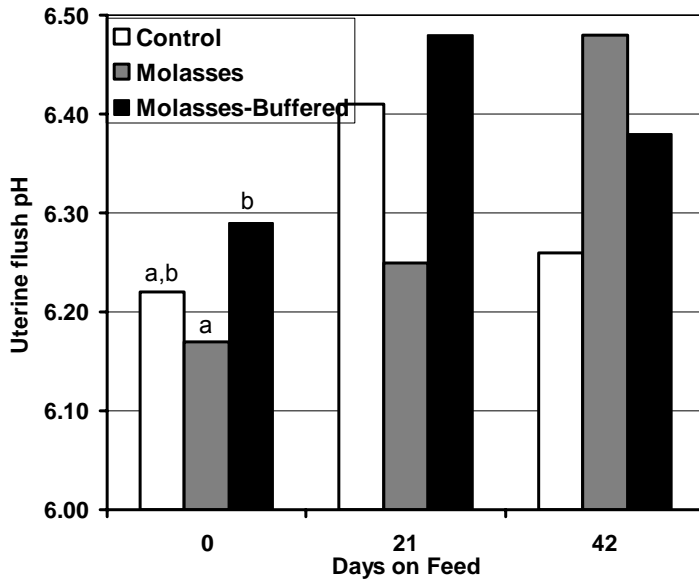


Figure 2. The effect of dietary cation-anion difference (DCAD) from different supplements on mature beef cow urine pH. Effect of treatment (day 0, $P=0.38$, $SEM=0.26$; day 21, $P<0.0001$, $SEM=0.17$; day 42, $P=0.002$, $SEM=0.12$).



^{a,b} Means with different superscripts differ, $P<0.05$

Figure 3. The effect of dietary cation-anion difference (DCAD) from different supplements on mature beef cow uterine pH. Effect of treatment (day 0, $P=0.02$, $SEM=0.03$; day 21, $P=0.29$, $SEM=0.11$; day 42, $P=0.69$, $SEM=0.12$).



^{a,b} Means with different superscripts differ, $P<0.05$