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Effect of Diets Containing Anionic Salts or Hydrochloric Acid Treated Alfalfa Silage on Blood and Urine Acid-Base Properties of Holstein Dry Cows

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Abstract: This trial was conducted to determine the effect of diets containing treated alfalfa silage with hydrochloric acid (HCl) (12 g kg^{-1} DM) or anionic salts (7.51% Sulfate, 29.8% Chloride, 11.49% Calcium and 2.98% Magnesium) on pH and mineral concentration (Ca, P, Cl, Mg, Na and K) of blood and urine of Holstein close-up dairy cows. In addition, milk yield and retained placenta after parturition (6 weeks) were recorded. Treatments were; Basal Diet (BD; corn silage (17 kg), alfalfa hay (2.4 kg), wheat straw (0.7 kg) and concentrate (5.5 kg)), HCl treated alfalfa silage (12 g kg^{-1} DM) substituted of alfalfa hay in basal diet (BDH) and basal diet + anionic salts mixture (BDA, 300 g/head/d). Experimental diets were fed to 12 Holstein dry cows (n = 4) during 18-33 days before calving. Animals were fed individually in tie stall as *ad libitum*. Feed intake was recorded daily and samples of blood (via jugular vein) and urine were taken at the pre and two weeks after the feeding. Samples were analyzed for pH and minerals concentrations. Results indicated that the effect of diets on urinary pH (7.06, 6.6 and 6.79 for treatments BD, BDH and BDA, respectively), blood pH and blood and urine minerals concentrations was not significant (p>0.05). Cows fed acidified diets had lower placenta excretion time (h) and more milk production than those of basal diet. It is concluded that blood and urine acid-base properties did not affected by the experimental diets when included by HCl or anionic salts.

Key words: Anionic salts, mineral of urine and blood, pH, dry cow

INTRODUCTION

Almost 50% of cows after parturition have a concentration of blood Ca <7 Mg dL^{-1}, therefore, these animals can not response to sudden requirement of Ca. Anionic diets with Dietary Cation-Anion Difference (DCAD) equal of -50 to -150 mEq kg^{-1} DM are used to feed in pregnant cows near to parturition for preventing milk fever (Block, 1984). Joyce *et al.* (1997) reported that the using of anionic diets caused to improve Ca homeostasis. In addition, compared with anionic salts, HCl as a readily available and inexpensive source of anions can be used for acidifying diet. Anionic diets fed at 14-21 days before parturition have been most effective in preventing milk fever when cows consumed excess of Ca (Goff and Horst, 1998).

Urine pH is often monitored to assess the degree of metabolic acidification resulting from the addition of anions to the diet (Charbonneau *et al.*, 2006). Therefore, pH of urine may be an inexpensive and sensitive method to monitor the effect of diet on acid-base property of animal and assess the risk of milk fever (Goff and Horst, 1998). Urine pH normally is 5.5-6.2 and pH <5.5 showed that anion content is more than cation and pH >8.2 indicated that anion content is less than cation (Horst *et al.*, 1967).

The objective of this study was to investigate the effect of Hcl 38% (used as alfalfa silage additive, 12 g kg^{-1} DM) or anionic salts (7.51% Sulfate, 29.8% Chloride, 11.49% Calcium and 2.98% Magnesium, 300 g/head/d) on mineral concentration, acid-base characteristics of blood and urine and first 6 weeks of milk production and retained placenta of close-up Holstein dry cows.

MATERIALS AND METHODS

Treatments: In the present study, 12 dry cows (18-33 days before parturition) were fed experimental diets including: BD: basal diet (corn silage (17 kg), alfalfa hay (2.4 kg), wheat straw (0.7 kg) and concentrate (5.5 kg)),

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Table 1: Chemical composition the close-up experimental diets

	Experimental diet[1]		
Item	BD	BDH	BDA
Crud protein (DM%)	14.5	15.1	14.2
Net energy lactation (Mcal kg^{-1} DM)	1.61	1.6	1.58
Net energy growth (Mcal kg^{-1} DM)	1.00	0.99	0.97
Neutral detergent fiber (DM%)	39.6	39.6	38.7
Forage neutral detergent fiber (DM%)	29.7	29.8	29
Acid detergent fiber (DM%)	24.8	25.2	24.2
Non-structural carbohydrates (DM%)	39.4	38.7	38.5
Metabolizable energy (Mcal kg^{-1} DM)	2.56	2.54	2.51
Ether extract (DM%)	2.6	2.7	2.5
Metabolizable protein (g d^{-1})	1193	1241	1194
Rumen degradable protein (g d^{-1})	1222	1273	1267
Rumen undegradable protein (g d^{-1})	584	623	585
Dry matter intake (kg DM d^{-1})	12.46	12.58	12.71
Minerals (%)			
Ca	1.1	0.89	1.45
P	1.08	1.01	1.06
Mg	0.63	0.58	0.73
Na	0.26	0.24	0.26
K	2.05	1.86	2
Cl	0.34	0.89	1.86
S	0.45	0.41	0.74

[1]BD: Basal Diet (corn silage (17 kg), alfalfa hay (2.4), wheat straw (0.7 kg) and concentrate (5.5 kg)); BDH: HCl treated alfalfa silage (12 g kg^{-1} DM) substituted of alfalfa hay in basal diet; BDA: basal diet + anionic salts mixture (300 g/head/d)

BDH: HCl treated alfalfa silage (12 g kg^{-1} DM) substituted of alfalfa hay in basal diet and BDA: basal diet + anionic salts mixture (300 g/head/d) using a completely random design. Animals were fed individually in tie stall as *ad libitum*. The composition of concentrate was; cottonseed meal 9.59%, barley grain 14.34%, corn grain 7.98%, wheat bran 3.71%, beet pulp 3.63% and D-calcium phosphate 0.4%. Chemical composition of the basal diet is given in Table 1. Cows were fed twice in 9:00 am and 21:00 pm and they had free access to drinking water.

Sampling: Feed intake was measured daily. Milk yield was recorded for 6 weeks after parturition while animals were fed diet containing 17% CP and 2.8 Mcal kg^{-1} DM. Time of excretion of placenta after parturition was recorded. Samples of blood were collected via jugular vein into heparinized tube glasses at 0.0 and 4 h after the morning feeding before and 2 weeks after the experimental period. Midstream urine samples were obtained at 4 h after the morning feeding in the same time of blood sampling, by manual stimulation of the vulva. Samples of blood were analyzed immediately for blood pH using blood gas analyzer system (AVL 995, Switzerland). Blood samples were centrifuged at 4000×g for 10 min to separate plasma and then were analyzed for mineral concentration (Ca, P, Cl, Mg, Na and K). Urine pH was immediately determined using a pH meter (Metrohm 1.691.0020, Swiss). A part of urine samples (10 mL) were acidified with 5 droplets of 2 N HCl. These samples were used to measure the mineral concentration. Na and K concentrations of blood and urine were determined using flame photometer (Jenway PFP Clinical Jenway Essex England) and Ca, P, Cl and Mg concentrations were determined by Auto-analyzer (Auto analyzer BT Targa 3000, Biotectica Rome, Italy).

Statistical analysis: Data was analyzed using the general linear models of SAS. Data of pre-experimental days were used as covariate to justify the model. Model was:

$$Y_{ij} = \mu + T_i + \beta (X_{ij} - X_{..}) + \varepsilon_{ij;}$$

where:

Y_{ij} = Dependent variable.
μ = Dependent variable mean.
T_i = Treatment effect.
β = Coefficient of covariate.
ε_{ij} = Residual error.

RESULTS AND DISCUSSION

Minerals concentrations and pH of urine are shown in Table 2. Treatments had not any effect on urine pH ($p>0.05$); however, urine pH of cows that consumed diet containing HCl was lower compared with the other animals. Acidified diets caused to stimulate mild metabolic acidosis in ruminant and increase excretion of H^+ in urine (Delaquis and Block, 1995). Reduction in blood pH, caused to stimulate Na^+/H^+ system in kidney medulary that excrete H^+ in urine (Oetzel *et al*., 1991). Goff and Horst (1998) found that the addition of HCl to the diet of non pregnant and non milking cows significantly reduced urine pH. In addition, Hu *et al*. (2007) and Charbonneau *et al*. (2006) reported that urine H^+ decreased linearly with increasing DCAD ($p<0.01$). Ca excretion in urine of animals fed diet containing HCl and anionic salts was higher than those fed BD. This finding confirmed the results of Jackson *et al*. (2001). Vagnoni and Oetzel (1998) observed that hypercalciurtic effect of anionic salts is likely due to the role of bone reabsorption in buffering a metabolic acid load. Cows were fed with acidic salts excreted about 6 g Ca in urine that a part of it might be in response to increase Ca transition from bone and increase Ca absorption from intestine (Vagnoni and Oetzel, 1998). Wang and Beed (1992) found an increase in urine Ca excretion in goats fed diets treated with HCl. Urine Ca content is an index of Ca balance in animals. The acidic diets might increase output of urine Ca, because of increase in intestinal absorption, increase rate of glumerular filtration and decrease Ca reabsorption from kidney tubules (Block, 1984). In the present study, there was no difference between treatments regarding P content of urine ($p>0.05$).

Table 2: Dry matter intake, pH and minerals concentration of urine of dry cows fed the close-up experimental diets

	Experimental diet[1]				
Item	BD	BDH	BDA	S.E.M	p-value
pH	7.06	6.60	6.79	0.42	0.80
Ca (mg dL^{-1})	8.83	13.97	17.40	5.57	0.45
P (mg dL^{-1})	1.60	0.58	1.54	1.07	0.08
Mg (mg dL^{-1})	16.04	22.22	30.47	7.57	0.11
Na (mEq L^{-1})	125.21	88.86	93.67	31.73	0.27
K (mEq L^{-1})	43.27	34.93	43.79	7.45	0.79
Cl (mEq L^{-1})	142.06	194.99	244.94	67.30	0.23
Dry matter intake (kg d^{-1})	12.37	12.55	12.52	0.01	0.40

[1]BD: Basal Diet (corn silage (17 kg), alfalfa hay (2.4), wheat straw (0.7 kg) and concentrate (5.5 kg)); BDH: HCl treated alfalfa silage (12 g kg^{-1} DM) substituted of alfalfa hay in basal diet; BDA: Basal Diet + Anionic salts mixture (300 g/head/d); S.E.M: Standard Error of Mean

Table 3: Mineral concentration and pH of blood of dry cows fed the close-up experimental diets

	Experimental diet[3]				
Item	BD	BDH	BDA	S.E.M	p-value
pH					
B[1]	7.44	7.33	7.34	0.03	0.39
A[2]	7.42	7.32	7.34	0.03	0.17
Ca (mg dL^{-1})					
B	7.65	7.79	8.36	0.49	0.45
A	7.94	8.21	8.45	0.48	0.25
P (mg dL^{-1})					
B	6.69	5.93	5.42	0.89	0.07
A	6.70	6.19	5.49	0.86	0.16
Mg (mg dL^{-1})					
B	2.18	2.21	2.34	0.14	0.26
A	2.11	2.23	2.48	0.27	0.06
Na (mEq L^{-1})					
B	130.62	136.5	130.60	3.18	0.02
A	138.32	137.4	135.52	3.16	0.10
K (mEq L^{-1})					
B	3.96	3.92	3.9	0.07	0.76
A	4.04	4.13	3.91	0.15	0.66
Cl (mEq L^{-1})					
B	97.420	98.70	101.53	2.82	0.02
A	100.98	101.29	102.22	1.06	0.56

[1]B: Before feed intake; [2]A: After feed intake; [3] BD: Basal Diet (corn silage (17 kg), alfalfa hay (2.4), wheat straw (0.7 kg) and concentrate (5.5 kg)), BDH: HCl treated alfalfa silage (12 g kg^{-1} DM) substituted of alfalfa hay in basal diet, BDA: Basal Diet + anionic salts mixture (300 g/head/d). S.E.M: Standard Error of Mean

However, cows fed the acidic diets (BDH and BDA) had the lowest urine P that was in agreement with the finding of Vagnoni and Oetzel (1998). Horst *et al.* (1967) reported that the increase in DCAD resulted in higher urine P, but, Joyce *et al.* (1997) observed the DCAD of diet had not impact urine P content. When Ca concentration of diet increased, intestinal absorption of P decreased and utilization of P in bone increased. Results of the present experiment showed that the diets had not effect on urine Cl and Mg content ($p>0.05$). This result might not confirm the findings of Vagnoni and Oetzel (1998). The lower urinary Mg concentration might be reflection of Mg absorption reduction because of the high Ca concentration in the diet (Oetzel *et al.*, 1991).

Diets containing high K concentration interfere with Mg ruminal absorption (Goff and Horst, 1997). In current study, there was not any difference between the treatments for urine Na content ($p>0.05$). Researchers showed that a reduction in DCAD caused to reduce Na excretion, also the increasing of Na in diet lead to elevate its excretion in urine (Oetzel *et al.*, 1991). In this study, urine K concentration was unaffected by HCl or acidic diets ($p>0.05$). Waterman *et al.* (1991) showed that the increase in urine K caused by the reflection of high k in diet.

Mineral concentration and pH of the blood of cows are shown in Table 3. Blood pH in cows fed diet containing HCl was less than the others. This result confirmed the data of Waterman *et al.* (1991). Blood pH is a major indicator of the ability of cows to maintain Ca homeostasis at calving (Goff and Horst, 1998). Present experimental diets did not affect on Ca concentration of blood of each sampling ($p>0.05$). However, cows fed diets containing HCl and anionic salts had higher Ca concentration than those fed BD. This result confirmed the findings of Oetzel *et al.* (1991) and Wang and Beed (1992). Reduction in blood pH (6.8-7.8) caused to increase blood Ca. The relationship between blood pH and ionized Ca might be explained by competition between ionized Ca and H^+ for serum protein binding sites when a cow is subclinically acidotic (Moore *et al.*, 2000). Low DCAD might increase blood Ca because anionic salts stimulate secretion of PTH hormone (Joyce *et al.*, 1997). Charbonneau *et al.* (2006) observed prepartum diets supplemented with acidogenic salts (low DCAD) increase plasma Ca concentration of cows at parturition. Results of this study showed that blood P concentration was not different between the treatments ($p>0.05$). High Ca concentration in diet caused to decrease absorption of P (Delaquis and Block, 1995). Blood Cl content of cows fed with BDH and BDA was higher than cows fed BD. Hu *et al.* (2007) reported that blood Cl was decreased linearly with increasing DCAD ($p<0.01$). Blood Mg concentration was not affected ($p>0.05$) by the current diets and confirmed the findings of Wang and Beed (1992). It has been reported that diet containing high Ca and K concentrations might interfere in Mg absorption (Delaquis and Block, 1995). Present experimental diets did not affect Na and K concentrations of dry cows blood ($p>0.05$). However, diet containing HCl increased blood Na. Roche *et al.* (2005) observed a quadratic increase in blood serum Na concentration and a linear decrease in blood serum K concentration in early lactating cows as DCAD increased.

Cows were fed diet containing HCl treated alfalfa silage had the highest non significant feed intake. Current

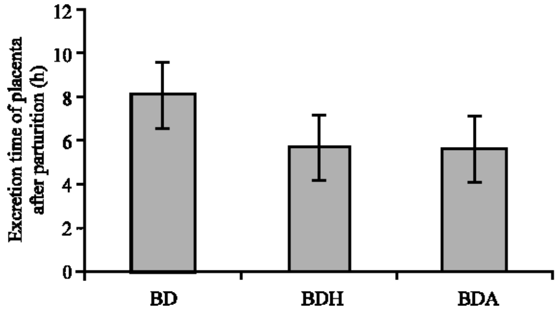

Fig. 1: Excretion time (h) of placenta after parturition of Holstein cows which fed close-up Experimental diets. BD: basal diet (corn silage (17 kg), alfalfa hay (2.4), wheat straw (0.7 kg) and concentrate (5.5 kg)); BDH: HCl treated alfalfa silage (12 g kg^{-1} DM) substituted of alfalfa hay in Basal Diet; BDA: basal diet + anionic salts mixture (300 g/head/d)

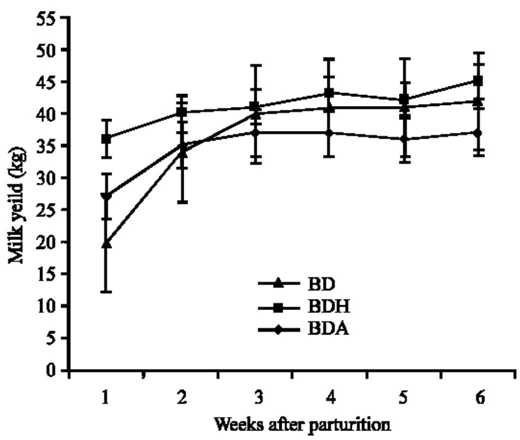

Fig. 2: Milk yield in the first 6 week of lactation of Holstein cows which fed close-up Experimental diets, BD: basal diet (corn silage (17 kg), alfalfa hay (2.4), wheat straw (0.7 kg) and concentrate (5.5 kg)); BDH: HCl treated alfalfa silage (12 g kg^{-1} DM) substituted of alfalfa hay in basal diet; BDA: basal diet + anionic salts mixture (300 g/head/d)

results confirmed the suggestion of Goff and Horst (1998). Anionic salts in diets fed to animal caused to decrease the palatability (Oetzel *et al*., 1991). While, diets containing equal amounts of Cl as HCl did not affect dry matter intake, even feed intake was increased, because of cows were adapted to it. Therefore, it is preferred to acidify the diets with HCl rather than anionic salts (Goff and Horst, 1997). Hu *et al.* (2007) concluded that dry matter intake increased linearly with increasing DCAD.

Time of placenta excretion (h) is shown in Fig. 1. Present results showed the excretion time of placenta after parturition was lower in animals fed anionic diets which confirmed the results of Goff and Horst (1998). The results of Joyce *et al*. (1997) showed, in cows fed low DCAD, incidence of retained placenta was zero, but Hu *et al.* (2007) observed diets with low DCAD did not alter the incidence of metabolic disorders.

Animals were fed diet containing HCl treated alfalfa silage had the highest milk yield in the first 6 weeks of lactation (Fig. 2). Moore *et al*. (2000), Hu *et al*. (2007) and Borucki Castro *et al*. (2004), reported DCAD of diets did not affect milk yield after postpartum, but Joyce *et al.* (1997) reported that while dry cow fed low DCAD diets (using anionic salts) had higher first 4 weeks milk production than non anionic diets fed cows.

CONCLUSION

Results of the present study showed that anionic salts or HCl (38%, 12 g kg^{-1} DM) treated alfalfa silage might be used in dry cow diets with some beneficial effects. Diets containing anionic salts caused to increase Ca excretion in urine and enhanced blood Ca concentration and decreased urinary pH. Cows fed acidified diets had lower placenta excretion time (h) and more milk production (first 6 weeks of lactation) compared with the animals fed non acidified diet.

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