Plasma mineral and energy metabolite concentrations in dairy cows fed an anionic prepartum diet that did or did not have retained fetal membranes after parturition

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Objective—To compare plasma total calcium, phosphorus, magnesium, nonesterified fatty acids (NEFA), beta hydroxy butyrate (BHB), and glucose concentrations in parturient dairy cows that were fed an anionic prepartum diet between those with and without retained fetal membranes (RFM) at 24 hours after parturition.

Animals—152 Holstein cows that calved during October through December of 1997.

Procedure—All cows were fed an anionic prepartum diet. Blood sample was taken within 6 hours after parturition from randomly selected cows. Thirty-nine cows had a diagnosis of RFM at 24 hours after parturition; 113 were not affected with RFM. At calving, body condition score (BCS; 1 [thin] to 5 [obese]), parity, and calving difficulty score were recorded. Plasma calcium, phosphorus, magnesium, NEFA, BHB, and glucose concentrations were compared between cows with or without RFM.

Results—Cows with RFM had significantly lower plasma calcium concentration soon after calving, compared with cows without RFM. Cows with a parity of ≥ 3 had significantly lower plasma concentrations of calcium and higher concentrations of magnesium, compared with cows with a parity of 1 or 2. Cows with a BCS of ≥ 3.25 at calving had significantly higher plasma concentrations of BHB than cows with a BCS of 2.75 to 3.0. Cows with dystocia had significantly higher plasma concentrations of glucose, compared with cows without dystocia.

Conclusions and Clinical Relevance—In parturient cows fed a prepartum anionic diet, those with RFM have lower plasma calcium concentrations than cows without RFM, although this association does not prove a cause-effect relationship. (*Am J Vet Res* 2004;65:1071–1076)

Retained fetal membranes (RFM) is a calving-related disorder with a reported incidence in dairy cattle of 4% to 11%.[1] Retained fetal membranes cause economic losses to the dairy industry as a result of treatment costs, increased incidence of metritis, lost milk production, increased risk of culling, and increased number of days between calving and subsequent pregnancy. The cost of each instance of RFM has been reported to range from $106 to $285.[2]

Retained fetal membranes is a complex disorder where several risk factors such as dystocia, parity, abnormal gestation length, season, and sire of the calf have been identified. Cows that develop parturient paresis are 4 times as likely to have RFM as cows without parturient paresis.[3] Subclinical hypocalcemia has been related to dystocia and RFM in dairy cattle as well.[3,4] Erb et al[5] determined that cows with hypocalcemia were 4.2 times and 2 times as likely to have dystocia and RFM, respectively. Risco et al[4] found that cows with RFM had lower plasma concentrations of calcium at parturition and up to 7 days after parturition than cows without RFM (6.27 ± 0.18 vs 7.40 ± 0.18 mg/dL, respectively). In the aforementioned studies, cows were not fed prepartum anionic salts.

In a recent study,[6] an immunologic mechanism for the pathogenesis of RFM is proposed. Fetal membranes must be recognized as foreign tissue and rejected by the immune system after parturition to cause their expulsion. Results of that study[6] revealed that a decrease in neutrophil function might be part of the cause of RFM. Cows that developed RFM had lower neutrophil chemotaxis and lower plasma interleukin-8 concentration before parturition than cows without RFM.[6] In addition, collagenase activity of cotyledon villi is involved in the process of releasing fetal membranes.[1] Collagenase activity is increased during delivery in healthy cows and decreased in cows with RFM.[1] Perhaps marginal plasma calcium concentrations are sufficient to reduce collagenase activity and alter the immune function, thereby resulting in a higher incidence of RFM.

Anionic salts have been shown to decrease the severity of hypocalcemia at calving in dairy cattle, but they do not prevent it completely.[7] Retained fetal membranes still is a prevalent condition in dairy cattle operations that feed anionic diets. The justification of anionic salts is to induce a mild acidification of the body, which enables the cow to mobilize calcium from bones and absorb calcium more efficiently from the intestine, thereby increasing the concentration of calci-

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um in blood.[8] The **dietary cation-anion difference (DCAD)** of a typical diet for lactating dairy cattle is between +100 to +250 mEq/kg of **dry matter (DM)**, using the equation DCAD (mEq/kg) = (Na + K) – (Cl + S). When anionic salts are fed during the prepartum period, this difference should be between 0 and -100 mEq/kg of DM.[7,8]

To our knowledge, the plasma concentration at or soon after calving of mineral and energy-related metabolites in dairy cows fed prepartum anionic salts and affected with RFM has not yet been determined. Because collagenases and motion of neutrophils are calcium-dependent processes, it is reasonable to hypothesize that even though anionic salts are fed, marginal hypocalcemia (calcium ≤ 7.5 mg/dL) at parturition might compromise collagenolysis and general neutrophil function, which in turn can increase the incidence of RFM. The purpose of the study reported here was to compare plasma total calcium, phosphorus, magnesium, **nonesterified fatty acids (NEFA)**, **beta hydroxy butyrate (BHB)**, and glucose concentrations in parturient dairy cows that were fed an anionic prepartum diet between those with and without RFM at 24 hours after parturition.

Material and Methods

Cows and herd management—The study reported here was conducted from June to December 1997 in a 3,600-cow commercial dairy farm located in north central Florida, with a rolling herd average for milk production of 10,500 kg. Cows were housed in a dry lot system and fed the same total mixed ration 3 times a day. During the transition period (21 days prior to expected calving until 21 days after parturition), cows received a diet higher in forage neutral detergent fiber (56.9% of neutral detergent fiber from forages) than the rest of the lactating cows in the herd (**Appendices 1 and 2**). Prepartum cows in the transition period (21 days before expected parturition) were housed in a separate dry lot and fed an anionic diet. The DCAD was -80 mEq/kg of DM as determined by use of the equation DCAD (mEq/kg) = (Na + K) – (Cl + S). The effectiveness of anionic salts was monitored through urine pH determination. Urine pH was determined once a week from a random subsample of 15% of the cows in advanced pregnancy (about 20 cows/wk).[8] Cows were separated and placed in a pen with several chutes. The escutcheon was stimulated to obtain a urine sample. When clean uncontaminated urine was observed, a sample was taken. Urine pH was determined immediately by use of a hand-held electronic pH meter.[a]

Experimental protocol—Considering a farm historical incidence of RFM of 20% and an expected difference in total plasma calcium concentration of 0.5 ± 0.4 mg/dL between cows with and without RFM, a minimum sample size of 30 cows/group was calculated (95% confidence; power of 80%). In 1997, 152 parturient cows that calved from October to December were randomly selected from a total of 700 parturient cows by a computer-generated table. Inclusion criteria included cows without parturient paresis or twins. Two blood samples were taken from the middle coccygeal vein of all cows by use of an evacuated tube.[b] One sample was taken in a heparinized tube to obtain plasma for all metabolite analyses except glucose, which was performed on plasma collected in sodium fluoride tubes. Samples were consistently collected within 6 hours after parturition and before any postpartum procedure or treatment was performed. During the entire transition period, cows were handled uniformly and exposed to the same environment. We made the assumption that any variability in sample collection time was equal between cows with and without RFM. Plasma calcium concentrations within 24 hours after parturition are stable.[7,8] Body condition at calving was scored by the same person according to a standardized method.[9] Parity and calving difficulty score (normal and dystocia [ie, forced traction]) were also recorded. Cows requiring fetotomy or cesarean section were excluded from the study.

The evaluation for RFM was conducted visually and vaginally by a veterinarian 24 hours after parturition.[1] Thirty-nine cows failed to expel their fetal membranes by 24 hours after calving and were considered to have RFM. One hundred and thirteen cows did not have RFM and were considered as controls. Outcome variables were the plasma concentrations of calcium, phosphorus, magnesium, NEFA, BHB, and glucose in cows affected or not affected by RFM.

Laboratory analysis—Immediately after collection, blood samples were centrifuged at 4,000 $\times$ *g* for 10 minutes. Plasma was separated and stored in plastic tubes and frozen at -20°C until analysis. Plasma samples were sent to the National Animal Disease Center, Agricultural Research Service, USDA for laboratory analysis.

Plasma mineral concentrations were evaluated by atomic absorption spectrophotometry (calcium and magnesium)[10] or colorimetrically (phosphorus).[11] Energy-related metabolites were evaluated by determination of NEFA, BHB, and glucose concentration in plasma. Plasma NEFA concentration was determined by use of an enzymatic-colorimetric method[12] with a commercial kit.[c] Plasma BHB concentration was determined by use of an enzymatic-colorimetric method[13] with a commercial kit.[d] Plasma glucose concentration was determined according to the method of Bergmeyer and Bernt[14] by use of a commercial kit based on the Trinder reaction.[e]

Statistical Analysis—Plasma concentrations of minerals, NEFA, BHB, and glucose of cows with RFM and clinically normal cows (controls) were analyzed by use of an ANOVA. A mixed linear model was developed because it implements random and fixed effects.[15,f] The model included the effect of group (RFM, yes or no), parity (1, 2, or ≥ 3), **body condition score (BCS)** at calving (scale 1 to 5 with increments of 0.25 points), dystocia (yes or no), and their respective double interactions. Least squares means were also reported. Values of $P \leq 0.05$ were considered significant. Goodness-of-fit of the models was performed by the Schwarz Bayesian criterion.[16]

The mixed-model ANOVAs were defined as follows:

$$y_{ijklm} = \mu + G_i + \text{cow}\,(G_i)_j + Par_j + BCS_k + Dys_l + (G \times BCS)_{ki} + (G \times Dys)_{li} + (BCS \times Dys)_{lk} + (Par \times G)_{ij} + e_{ijklm}$$

where, y_{ijklm} = blood metabolite concentration, G_i= fixed effect of group (RFM, yes or no), cow $(G_i)_j$ = random effect of cow nested in group, Par_j = fixed effect of parity, BCS_k = random effect of BCS at calving, Dys_l = random effect of dystocia, $(G \times BCS)_{ki}$ = random effect of interaction group and BCS, $(G \times Dys)_{li}$ = random effect of interaction group and dystocia, $(BCS \times Dys)_{lk}$ = random effect of interaction BCS and dystocia, $(Par \times G)_{ij}$ = fixed effect of interaction parity and group, and e_{ijklm} = random error term.

Results

Minerals—No significant difference was found in the number of days that the prepartum diet was fed between cows with RFM (20.9 ± 1.7 days) and cows without RFM (20.1 ± 1.8 days). Urine pH ranged from 6.2 to 7.2 during the time the trial was conducted, indicating that the diet and the time cows were consuming anionic salts were successful in inducing a metabolic acidosis. The reference range value for urine is a pH of > 8.0 when cows are fed a diet with a positive DCAC.[7,8] Anionic salts change the acid-base balance in < 4 days; therefore, homeostatic mechanisms take place in a short period.[7,8]

Only RFM and parity were associated with plasma calcium concentrations at parturition (**Table 1**). Cows with a diagnosis of RFM at 24 hours after parturition had significantly lower plasma calcium concentrations soon after calving than cows without RFM. Cows with a parity of ≥ 3 had lower plasma calcium concentrations than cows with parities of 1 and 2. Retained fetal membranes, parity, and dystocia had no association with plasma phosphorus concentrations at parturition. Retained fetal membranes and dystocia were not associated with plasma magnesium concentrations. Only parity had a significant association with plasma magnesium concentrations at parturition. Cows with a parity of ≥ 3 had significantly higher concentrations of magnesium than cows with parities of 1 and 2. Only 32.3% of cows had plasma calcium concentrations that were indicative of subclinical hypocalcemia (< 7.5 mg/dL). Cows with subclinical hypocalcemia were 1.72 (95% confidence interval, 0.8 to 3.9) times as likely to develop RFM as cows with plasma calcium concentrations of calcium > 7.5 mg/dL.[8] Similarly only 33.0% of cows had plasma concentrations of phosphorus that were < 3.5 mg/dL, and only cows of parity ≥ 3, as with calcium status, had a low mean concentration of phosphorus (3.5 mg/dL). No significant double interactions for minerals were found in models of this study.

Energy-related metabolites—No association was found between RFM and any of the energy-related metabolites (NEFA, BHB, glucose; **Table 2**). Parity and BCS were associated with plasma BHB concentrations at parturition. Cows with dystocia had significantly higher concentrations of glucose than cows calving unassisted. Cows with dystocia had higher plasma concentrations of BHB than cows calving unassisted, but this difference was not significant (P = 0.09). Cows with parities of 2 or ≥ 3 had higher plasma concentrations of BHB than cows with a parity of 1. Cows with BCS ≥ 3.25 had significantly higher plasma concentrations of BHB than cows with lower BCS. No significant double interactions for energy-related metabolites were found in models of this study.

Table 1—Least square means (± SEM) plasma mineral concentrations of parturient cows according to retained fetal membrane status, parity, and dystocia status.

		Plasma mineral concentrations		
Variables	No. of cows	Calcium (mg/dL)	Phosphorus (mg/dL)	Magnesium (mg/dL)
RFM	39	7.17 ± 0.36*	3.73 ± 0.25	2.03 ± 0.08
No RFM	113	8.76 ± 0.28	4.00 ± 0.22	2.00 ± 0.07
Parity = 1	25	8.38 ± 0.31†	4.19 ± 0.33	1.83 ± 0.09†
Parity = 2	43	7.97 ± 0.26†	3.87 ± 0.24	1.99 ± 0.07†
Parity ≥ 3	84	7.55 ± 0.25	3.52 ± 0.23	2.23 ± 0.07
Dystocia	38	7.89 ± 0.32	3.97 ± 0.25	2.01 ± 0.09
No dystocia	114	7.82 ± 0.40	3.74 ± 0.24	2.12 ± 0.08

*Significantly ($P < 0.05$) different from cows without RFM.
†Significantly ($P < 0.05$) different from cows of parity ≥ 3.
RFM = Retained fetal membranes.

Table 2—Least square means (± SEM) plasma energy metabolite concentrations of parturient cows according to retained fetal membrane status, parity, dystocia status, and body condition score.*

		Plasma energy metabolite concentrations		
Variables	No. of cows	NEFA (mEq/L)	BHB (mg/dL)	Glucose (mg/dL)
RFM	39	0.95 ± 0.08	6.22 ± 0.54	66.1 ± 2.42
No RFM	113	1.07 ± 0.07	6.31 ± 0.47	62.3 ± 2.17
Parity = 1	25	0.94 ± 0.11	5.13 ± 0.67†	64.3 ± 3.31
Parity = 2	43	1.03 ± 0.07	6.97 ± 0.55	63.6 ± 2.41
Parity ≥ 3	84	1.06 ± 0.07	6.71 ± 0.54	64.7 ± 2.18
Dystocia	38	1.03 ± 0.09	6.81 ± 0.52	67.1 ± 2.29‖
No dystocia	114	0.99 ± 0.09	5.73 ± 0.51	61.3 ± 2.23
BCS of 2.75	5	0.91 ± 0.18	4.06 ± 1.21‡	60.8 ± 5.67
BCS of 3.00	24	1.17 ± 0.10	5.96 ± 0.52§	65.8 ± 2.68
BCS of 3.25	52	1.00 ± 0.07	6.65 ± 0.41	65.7 ± 1.93
BCS of 3.50	53	0.94 ± 0.08	7.63 ± 0.48	67.2 ± 2.29
BCS of 3.75	14	1.10 ± 0.13	6.38 ± 0.72	65.0 ± 3.51
BCS of 4.00	4	0.93 ± 0.28	6.97 ± 1.85	60.8 ± 7.27

*Body condition scores were as follows: 1= thin, 5= obese.
†Significantly ($P < 0.05$) different from cows of parities 2 or ≥ 3.
‡Significantly ($P < 0.05$) different from cows with a BCS of ≥ 3.25.
§Significantly ($P < 0.05$) different from cows with a BCS of 3.5.
‖Significantly ($P < 0.05$) different from cows with no dystocia.
NEFA = Nonesterified fatty acids. BHB = Beta hydroxy butyrate.
BSC = Body condition score.

Discussion

In our study, cows with a diagnosis of RFM at 24 hours after parturition and that had been fed anionic salts before parturition had lower plasma calcium concentrations at or soon after parturition than cows without RFM. However, the mean plasma calcium concentration of the entire group in our study was > 7.5 mg/dL, suggesting that the proportion of cows (32.3%) with hypocalcemia (< 7.5 mg/dL), as defined by Goff et al,[17] in our study herd was low, which is likely a result of the use of an anionic prepartum diet (negative DCAD). The finding of urine pH in the prepartum cows that was between 6.2 and 7.2 supports this observation. Also only 2 instances of clinical hypocalcemia (parturient paresis) were found within the entire population of cows calving (n = 700) during the study period. The association between RFM and low plasma calcium concentrations has been reported in other studies[4,18]; however, these cows were not fed anionic salts before parturition. The mean plasma calcium concentration at calving in cows with or without RFM in 1 study[4] was 5.4 and 6.8 mg/dL, respectively. These values were almost 1.5 mg/dL lower than the values found in our study. Although cows with RFM in our study were receiving anionic salts, they still had lower plasma calcium concentrations soon after calving than cows without RFM. This low concentration might be affecting the mechanism of detachment of cotyledon from uterine caruncles. Collagenase activity of cotyledon villi is involved in the process of releasing of fetal membranes.[1] Collagenase (a calcium-dependent enzyme)[19] activity is increased during delivery in healthy cows and decreased in cows with RFM.[1] Perhaps marginal calcium concentrations are sufficient to reduce collagenase activity and alter the immune function, thereby causing a higher incidence of RFM. Indeed, new evidence has been found[6] that indicates that the pathogenic process leading to RFM starts before calving and the clinical expression of RFM occurs at 24 hours after parturition. In that study,[6] it was postulated that fetal membranes must be recognized as foreign tissue and rejected by the immune system after parturition to cause expulsion of the membranes. The authors[6] found that a decrease in neutrophil function might be part of the cause of RFM in dairy cattle. They also found that cows that had RFM had lower neutrophil chemotaxis and lower plasma interleukin-8 concentration than cows without RFM. Interleukin-8 is a potent neutrophil chemoattractant, which induces a vicious cycle for the process of chemotaxis.[20,21] In addition, 1 of the normal functions of neutrophils is the release of proteases and collagenases from secondary granules. Conversely, the chemotaxis activity and neutrophil movements are determined by the action of a microfilament system.[21] Because collagenases and motion of neutrophils are calcium dependent processes,[22] it is reasonable to assume that marginal hypocalcemia (calcium concentrations of ≤ 7.5 mg/dL) at, or just before, parturition might compromise uterine contractions, collagenolysis, proteolysis, and general neutrophil function, which in turn can increase the incidence of RFM.

Because the pathogenic process leading to RFM is initiated before parturition[6] and we determined plasma calcium concentration soon after calving, the plasma concentrations of this mineral just after parturition cannot be considered a risk factor for RFM. Consequently the relationship between these 2 variables has to be addressed as a simple association. In our study, cows of parity ≥ 3 had lower plasma concentrations of total calcium than young cows. This relationship between age and plasma calcium concentrations has been well documented.[23,24]

In contrast to findings in previous reports,[3,4,5] cows in our study with or without dystocia had similar plasma calcium concentrations. This inconsistency among study results might be explained by the sample size in our study, which might have decreased the power to detect significant differences in plasma calcium concentrations between cows with or without dystocia (312 cows/group are needed).

Cows that develop hypocalcemia have blood phosphorus concentrations that are even further decreased.[7] This relationship between plasma calcium and phosphorus concentrations was partially found in our study. Plasma calcium concentrations were < 7.5 mg/dL in 32.3% of cows; similarly 33.0% of cows had plasma concentrations of phosphorus that were < 3.5 mg/dL. Only cows of parity ≥ 3, as with calcium status, had a low mean plasma concentration of phosphorus (3.5 mg/dL). Cows of parity ≥ 3 had higher plasma concentrations of magnesium than young cows (2.2 ± 0.02 vs 1.80 ± 0.02 mg/dL). This might be the result of old cows having lower plasma calcium concentrations than young cows. This would increase the blood parathyroid hormone concentrations and decrease the urinary excretion of magnesium.[17] Goff and Horst[8] reported a similar pattern for plasma concentrations of magnesium, although plasma concentrations of magnesium were higher at calving (mean, 2.6 mg/dL) in that study than in our study.

Because cows with high BCS at calving (> 3.5) and dystocia had higher plasma concentrations of BHB, overfeeding during the nonlactating period is not recommended. Fat cows eat less, have higher plasma concentrations of NEFA at parturition, and increase BHB synthesis.[25,26] Conversely, parturition and dystocia are characterized by high blood concentrations of corticosteroids and cathecolamines.[27] As a result, both hormones enhance the rate of lipolysis, glycogenolysis, and gluconeogenesis, thereby increasing the concentrations of glucose and NEFA in blood.[28,29]

Cows that had dystocia had higher plasma concentrations of glucose than cows calving unassisted. The stress induced by dystocia may possibly increase blood catecholamine concentrations, thereby stimulating gluconeogenesis and fatty acid mobilization.[8] Furthermore, glucocorticosteroids could enhance the fat-mobilizing effects of other hormones. Both hormones can increase the plasma concentration of glucose in stressed ruminants.[30]

Body condition score at calving was significantly associated with the plasma concentration of BHB. Cows with higher BCS (≥ 3.25) at calving had significantly higher plasma concentrations of BHB than cows with BCS of 2.75 to 3.0. The biological importance of this finding is questionable because BHB concentrations < 8.0 mg/dL are considered to be within the reference range for cows at calving.[31,32] Only 3.3% (5/151) of cows had plasma BHB concentrations indicative of subclinical ketosis (> 1.4 mmol/L).[31,32] Low prevalence of subclinical ketosis was expected because blood samples were taken soon after calving. The risk period for an increased incidence of subclinical ketosis starts about 7 days after parturition.[32] In addition, cows with parities of 2 or ≥ 3 had significantly higher plasma concentrations of BHB than young cows. Cows of parity ≥ 3 may produce more milk and require more metabolites such as glucose or amino acids to synthesize milk components and are at higher risk of developing subclinical ketosis.[32] Additionally, heavier cows at calving have both the highest plasma BHB concentrations at and after calving and are at higher risk of developing hyperketonemia, compared with cows with a moderate and low BCS.[32-34]

In conclusion, although mean plasma calcium concentration of the entire study population was > 7.5 mg/dL, cows fed an anionic prepartum diet and that had a diagnosis of RFM at 24 hours after parturition still had lower concentrations of total plasma calcium at or soon after calving (< 7.2 mg/dL) than cows without RFM. Phosphorus, magnesium, and energy-related metabolites were not associated with the condition of RFM. Cows of parity ≥ 3 had low plasma calcium concentrations and high plasma magnesium and BHB concentrations. Dystocia was associated with high plasma concentrations of glucose. Cows of parity ≥ 2 and those with BCS ≥ 3.25 had high plasma concentrations of BHB. The association between plasma calcium concentrations and the condition of RFM did not reveal a cause and effect relationship.

[a]Micro pHep3 waterproof pH tester, Hannah Instruments, Woonsocket, RI.
[b]Vacutainer, Becton Dickinson, Rutherford, NJ.
[c]NEFA-C kit WAKO, Wako Chemicals Inc, Richmond, Va.
[d]Sigma beta-BHA kit #310–A, Wako Chemicals Inc, Richmond, Va.
[e]Sigma Chemical Co, St. Louis, Mo.
[f]PROC MIXED, SAS Institute Inc, Cary, NC.

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Appendices continued on the next page

Appendix 1

Feed composition of the prepartum transition diet fed for 21 days before expected parturition.

Feed composition	Dry matter (%)
Alfalfa hay early vegetative	10.0
Coastal hay, Florida	6.1
Corn silage	7.4
Sorghum silage	6.0
Wet beet	21.8
Citrus pulp	10.1
Cottonseed whole lint	10.1
Ammoniated whey*	3.5
Corn hominy	13.5
Energy supplement†	3.6
DCAD supplement‡	4.8
Soybean meal§	3.1

*Ammoniated whey contained 61.5% crude protein.
†Composed of sodium propionate, propylene glycol, dried whey, and calcium carbonate.
‡Anionic salts, mineral, and vitamin supplement for nonlactating cows in advanced pregnancy during the transition period.
§Soybean meal contained 48% protein.
DCAD = Dietary cation-anion difference.

Appendix 2

Nutrient content of the prepartum transition diet.

Nutrient	Values
CP (% of DM)*	16.83
Undegradable Protein (% of CP)†	38.77
Degradable Protein (% of CP)†	61.23
Soluble Protein (% of CP)†	34.69
Net energy of lactation (Mcal/kg of DM)†	1.67
ADF (% of DM)*	23.55
NDF (% of DM)*	34.65
Forage (% of NDF)†	56.89
Nonstructural carbohydrates (% of DM)*	31.94
Lipid (% of DM)*	4.93
Ash (% of DM)*	8.68
Calcium (% of DM)*	1.28
Phosphorus (% of DM)*	0.35
Magnesium (% of DM)*	0.38
Potassium (% of DM)*	1.07
Sodium (% of DM)*	0.11
Chloride (% of DM)*	0.51
Sulfur (% of DM)*	0.33
DCAD (mEq/kg of DM)‡	–80

*Values obtained from chemical analysis.
†Values obtained from nutritional composition tables.
‡Values obtained from the following formula: DCAD (mEq) = (Na + K) – (Cl + S) after wet chemistry analysis of the ingredients.
CP = Crude protein. DM = Dry matter. ADF = Acid detergent fiber. NDF = Neutral detergent fiber.