Interim report

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Gregory Penner
Department of Animal and Poultry Science
College of Agriculture and Bioresources, University of Saskatchewan
51 Campus Drive
Saskatoon, SK. S7N 5A8
greg.penner@usask.ca
306-966-4219

HQP TRAINING

The project to date has supported the training of 1 PhD student (Kasia Burakowska). Technical support was provided by Gillian Gratton.

DISSEMINATION

- Research results have been presented at the Western Canadian Dairy Seminar (2018) in Red Deer
- Research results have been presented at the Canadian Society of Animal Science Annual Meeting (2018) in Vancouver

INTRODUCTION

Although canola meal is commonly used as a protein source in rations for dairy cows and beef cattle, its use in rations for newborn calves is limited with soybean meal still being the preferred protein source in starter mixtures. This limited use of canola meal for calves are mainly results of concerns with low palatability and low digestibility of canola meal when offered to calves (Fiems et al., 1985; Khorasani et al., 1990). Low palatability and digestibility negatively affect solid feed intake and performance of animals, especially after weaning; a time when it is critically important to maximize dry matter intake. However, we have shown that inclusion of glycerol promotes the starter intake for calves fed a canola-protein based starter mixture (Burakowska et al., 2018). In addition, Hadam et al., (2018) study demonstrated that the use of canola meal as 50% of the protein source does not negatively affect starter mixture intake, ADG, or gain:feed when other dietary factors (e.g. glycerol) that improve palatability are included in the diet. While those study demonstrated canola meal is acceptable, including canola meal as the primary protein source had negative effects on ADG and gain:feed relative to diets with soybean meal.

Our hypothesis was that including canola meal in the starter mixture by replacing up to 60% of the soybean meal would not affect calf performance before and after weaning. The objective of this study was to determine the optimal inclusion rate of the canola meal in the dairy calf starters.

MATERIALS AND METHODS

Animals, Housing and Feeding Regiment. A total of 50 new-born heifers (birth weight 39.8 ± 3.4 kg, mean ± SD) at the Rayner Dairy Research and Teaching Facility were enrolled into this study. Heifers were blocked by birth date and within block randomly assigned to 1 of 5 treatments differing in the canola meal (**CM**) inclusion rate. The basal pelleted starter mixture contained: barley grain; corn grain; soybean meal; wheat bran; glycerol; molasses; urea; minerals and vitamins targeting 21% CP. To evaluate the effect of canola meal inclusion, CM was incorporated into the diet to provide 0, 15, 30, 45 and 60% of the crude protein level originally supplied by soybean meal. Crude protein and starch concentrations were balanced across treatments with corn, barley, corn gluten meal, and wheat bran (Table 1).

Calves were housed individually in pens (1.5 × 2 m) with wood shavings as bedding in the calf barn. Calves were weighed at birth and weekly thereafter (each Tuesday at 1000 h). Body weights were used to assess average daily gain and to adjust the amount of milk replacer provided targeting 15% of BW (milk replacer was mixed using 150 g DM milk replacer powder/L milk). The amount of milk replacer consumed and refused was recorded per feeding throughout the study. Calves were fed 3 times per day (at 0600, 1500, 2100 h) until 35.3 ± 2.4 d of age. Subsequently calves were exposed to a 3-wk step-down weaning process with the amount of milk replacer reduced to 10% of BW from d 36 to 42, to 7% BW from d 43 to 49 with milk replacer provided in 2 feedings (at 0600, 2100 h). Starting on d 50 until d 56, calves received milk replacer at 3% of BW offered during the morning feeding (0600 h) with no further milk replacer provided after d 57.

Calves received their respective starter mixture treatment at 8 d of age with the amount of starter offered and refused recorded daily. Calves were provided sufficient starter to ensure that achieved voluntary starter intake. Fresh starter was provided daily at 0700 h. No forage was fed during this study to avoid confounding effects of forage intake. Corresponding with provision of the starter, fresh water was available ad libitum. Samples of the starter mixture and milk replacer powder were collected weekly and dried at 55°C for 72 h. They were composited monthly and ground on 1 mm sieve (Christy and Norris, Christy Turner Ltd., Chelmsford, UK). They were sent to Cumberland Analytical for chemical analysis of: DM, ash, CP, NDF, ADF, starch, water soluble carbohydrates, ether extract, calcium and phosphorus. Dry matter was analyzed using method 930.15 by drying the sample at 135°C (AOAC, 2000). Crude protein was analyzed using method 990.03 (AOAC, 2000) using Leco FP-528 Nitrogen Combustion Analyzer (Leco, St. Joseph, MI). Acid detergent fibre was analyzed using method 973.18 (AOAC, 2000) with modifications where Whatman 934-AH glass microfiber filters with 1.5 µm particle retention were used. Neutral detergent fiber was analyzed as described by Van Soest et al. (1991) with the same modification as ADF method. Starch was analyzed according to Hall (2009). Ash was analyzed with method 942.05 (AOAC, 2000), with 1.5 g sample weight, 4 h ash time and hot weight being used. Fat was analyzed using method 2003.05 (AOAC, 2006) using Tecator Soxtec System HT 1043 Extraction unit (Tecator, Foss, Eden Prairie, MN). Mineral analysis was conducted using method 985.01 (AOAC, 2000) using Perkin Elmer 5300 DV ICP (Perkin Elmer, Shelton, CT).

On d 62.2 ± 0.8 (6.2 ± 0.8 d post weaning, mean ± SD), jugular catheters were inserted, and blood samples were collected on d 63.2 ± 0.8 every 4 hours (6 samples in total) at 0600, 1000, 1400, 1800, 2200, 0200 h for measurement of plasma urea nitrogen, BHBA, glucose, and insulin. Plasma insulin concentration was analyzed using Mercodia Bovine Insulin ELISA kit (Mercodia, Uppsala, Sweden). β-hydroxybutric acid concentration in serum was measured based on the method described by Williamson et al. (1962). Plasma urea concentration was analysed using method by Fawcett and Scott (1960). Plasma glucose concentration was analysed by enzymatic reaction of the present glucose with glucose oxidase/peroxidase (No. P7119 Sigma, St. Louis, MO) and dianisidine dihydrochloride (No. D3252 Sigma, St. Louis, MO) in a 96-well plate and absorbance measure at 450 nm wavelength using a plate reader (Epoch 2, BioTek Instruments, Inc., Winooski, VT).

On d 66.2 ± 0.8, total tract digestibility measurements were initiated. In preparation, calves were weighed on 2 consecutive days (d 64 and 65) prior to the start of the measurements. The calf pellet contained 0.2% titanium oxide to facilitate the digestibility measurements (4 d of collection from d 66 to 69). Fecal samples were collected every 12 h, with 3 h offset daily, therefore on d 66 samples were collected at 0300 and 1500 h, on d 67 at 0600 and 1800 h, on d 68 at 0900 and 2100 h and on d 69 at 1200 and 0000 h. A total of 8 samples were collected. At each timepoint, 100 g of feces were collected directly from the rectum. Samples were pooled and frozen until further analysis of dry matter and chemical composition as described for feed samples. Calf BW was determined for 2 consecutive days after completion of the digestibility measurements (d 70 and 71). Titanium concentration in both feed and fecal samples was measured according to the method by Myers et al. (2004), which includes digestion of samples (Tecator Digestor Auto 1011 3844, FOSS) with 98% sulfuric acid in the presence of catalyzer (FisherTab™ CT-37 Kjeldahl Tablets K3011000, Fisher Scientific) for 2 h, addition of 30% hydrogen peroxide, filtration of samples and absorbance measurement at 410 nm. Afterwards, nutrient digestibility was calculated as follows:

$$Nutrient\ digestibility\ (\%) = 100 - 100 \times \left(\frac{TiO_{2feed}\ (\%)}{TiO_{2feces}\ (\%)} \times \frac{Nutrient_{feces}(\%)}{Nutrient_{feed}\ (\%)}\right)$$

Ruminal Fermentation Characteristics. On 70.2 ± 0.8 d of age (13.2 ± 0.8 d after weaning, mean ± SD), ruminal fluid was collected 6 h post feeding (1300 h) to evaluate ruminal short-chain fatty acid (**SCFA**) and ammonia concentrations. Ruminal fluid was sampled through an esophageal tube

connected to a pressure pump (DOA-P704-AA, Gast Manufacturing, Inc., Benton Harbor, MI) with a digital timer (H5CX, OMRON, Tokyo, Japan). Two 10 mL samples were collected, the first one was mixed with 2L of meta-phosphoric acid (25% wt/vol) and the second one was mixed with 2 mL od 1% sulfuric acid. If the whole volume of 10 mL of the ruminal fluid was not collected, the amount collected was recorded to be later included in the calculations as dilution factor. Both samples were frozen at -20°C until further analysis. Short chain fatty acid concentration was analyzed using gas-chromatography (Agilent Technologies Inc., Santa Clara, CA) as described by Khorasani et al. (1996), while ammonia concentration was analyzed using the method detailed by Fawcett and Scott (1960).

Statistical Analysis. Data were analyzed as a randomized complete block design including treatment as fixed effect and block as random effect. Polynomial contrasts were used to evaluate the linear, quadratic and cubic responses to increasing canola meal inclusion in the diets. For variables collected over time, week of measurement (when relevant) was included in the model as fixed effect and treated as a repeated measure. Under the latter scenario, the 2-way interaction between treatment and time was assessed. Significance was declared when $P \leq 0.05$ and tendency was declared when $0.05 < P \leq 0.10$.

RESULTS

As per design, the starters were similar in the CP content (Table 2), although ADF and NDF content numerically increased with the canola meal inclusion, while water soluble carbohydrates numerically decreased.

Dry matter apparent total tract digestibility was not affected by increasing canola meal inclusion rate ($P \geq 0.23$, Table 3). Apparent total tract digestibility of crude protein in the starters responded cubically to the increasing canola meal inclusion ($P = 0.010$), decreasing from 0 (73.0%) to 15 (70.7%), increasing for 45 (72.5%) and decreasing again for 60 (70.0%). Starch apparent total tract digestibility tended to respond cubically ($P = 0.062$), decreasing from 0 (98.5%) to 15 (98.1%), then increasing until 45 (99.2%) and decreasing for 60 (98.8%). Ether extract apparent total tract digestibility responded cubically as well ($P = 0.011$), decreasing from 0 (67.1%) to 15 (65.2), increasing for 30 (74.4%) and decreasing until 60 (65.0%).

Milk replacer intake did not differ between treatments ($P \geq 0.32$, Table 4). Starter intake between d 8 until d 35 of age did not differ between treatments ($P \geq 0.17$), while the overall (d 8 to 71, $P = 0.055$), step-down (d 36 to 56, $P = 0.063$), and post-weaning (d 57 to 71, $P = 0.053$) starter intake tended to result in a linear decrease with increasing CM concentration of 0.158, 0.222, 0.337 kg respectively between 0 and 60 treatments. Overall, step-down and post-weaning ADG did not differ among treatments ($P \geq 0.12$). Average daily gain between d 8 to 35 responded cubically to increased canola meal inclusion ($P = 0.008$), increasing from 0 (0.768 kg/d) to 15 (0.823 kg/d) and decreasing for 45 (0.750 kg/d) and increasing for 60 (0.801 kg/d). Initial and final BW, cumulative growth and post weaning feed efficiency were not affected by treatment ($P \geq 0.22$).

Ruminal pH was not affected by CM inclusion rate ($P \geq 0.64$, Table 5). We observed a tendency ($P = 0.083$) for treatment difference in short-chain fatty acid concentration, without clearly defined CM effect. Molar proportion of acetate responded cubically to the increased canola meal inclusion level in the starters ($P = 0.044$), decreasing from 0 treatment (47.6 mol/100 mol) to 30 (46.5 mol/100 mol), increasing for 45 (48.9 mol/100 mol) and decreasing for 60 (43.8 mol/100 mol). Increasing level of canola meal in starters tended to linearly increase the propionate molar proportion by 4.9 mol/100 mol ($P = 0.075$). Iso-butyrate molar proportion tended to respond

cubically to the increased canola meal inclusion ($P = 0.085$). Increased canola meal rate within the starters caused butyrate to respond cubically ($P = 0.021$), increasing from 0 treatment (8.54 mol/100 mol) to 15 (10.18 mol/100 mol), decreasing until 45 (5.81 mol/100 mol) and increasing until 60 treatment (9.41 mol/ 100 mol). Iso-valerate molar proportion did not differ among treatments ($P = 0.24$). Valerate molar proportion decreased linearly from 3.73 mol/100 mol for 0 treatment to 3.23 mol/100 mol for 60 treatment ($P = 0.034$). Caprate molar proportion responded cubically to increased canola meal inclusion, decreasing from 0 treatment (1.30 mol/100 mol) to 15 (0.73 mol/100 mol), increasing for 30 (1.19 mol/100 mol) and deceasing until 60 (0.83 mol/100 mol). With the increasing canola meal concentration, we observed a cubic response for ammonia concentration ($P = 0.025$), with the value decreasing from 0 treatment (4.00 mg/dL) to 15 (3.00 mg/dL), increasing until 45 treatment (5.05 mg/dL) and decreasing for 60 (3.42 mg/dL).

Plasma glucose concentration did not differ among treatments ($P = 0.46$, Table 6). Plasma insulin concentration responded cubically to increased CM inclusion in the starter ($P = 0.045$), decreasing from 0 treatment (0.549 µg/L) to 45 (0.403 µg/L) and increasing for 60 (0.594 µg/L). β-hydroxybutyrate plasma concentration tended to respond cubically to increasing CM rates in starter feed ($P = 0.067$), increasing the value from 0 treatment (0.218 mmol/L) to 15 (0.251 mmol/L), decreasing until 45 (0.187 mmol/L) and increasing until 60 (0.236 mmol/L). Plasma urea concentration linearly decreased with increasing CM inclusion rate from 13.2 mg/dL for 0 treatment to 12.0 mg/dL for 60 treatment ($P = 0.044$). We did not observed treatment by time interactions for any of the analyzed blood metabolites ($P \geq 0.28$, Table 7); however, for glucose ($P < 0.001$), insulin ($P = 0.023$) and BHBA ($P < 0.001$) differences were observed among hours of sampling (Table 8). Glucose concentration was the greatest at 0200h (64.0 mg/dL) and the least at 1000h (60.3 mg/dL). Insulin concentration was the greatest at 0200h (0.703 µg/L) and the least at 0600h (0.438 µg/L). β-hydroxybutyrate concentration was the greatest at 0200, 1400 and 1800h (0.222, 0.222 and 0.228 mmol/L respectively) and the least at 0600h (0.186 mmol/L).

IMPLICATIONS

Use of canola meal in starters for calves by replacing 45% of the crude protein from soybean meal does not negatively affect starter intake, average daily gain, or post-weaning feed efficiency. This study further supports the potential to utilize canola meal in diets for calves prior to and post-weaning.

Table 1. Ingredient composition of the pelleted calf starters differing in canola meal inclusion rate: 0, 15, 30, 45 and 60% of the CP originally supplied by soybean meal.

Ingredient (% DM)	Treatment				
	0	**15**	**30**	**45**	**60**
Soybean meal	28.4	24.1	19.8	15.7	11.4
Canola meal	0.0	5.2	10.4	15.7	20.7
Barley grain	15.8	17.7	18.0	18.7	20.7
Corn grain	21.8	21.4	22.3	22.8	22.5
Corn gluten meal	1.0	1.5	2.0	2.5	3.0
Wheat bran	21.1	18.1	15.7	12.7	9.8
Salt	0.5	0.5	0.5	0.5	0.5
Limestone	2.2	2.2	2.2	2.2	2.2
Molasses	2.2	2.2	2.2	2.2	2.2
Mineral supplement[1]	1.1	1.1	1.1	1.1	1.1
Whey protein	2.6	2.6	2.6	2.6	2.6
Glycerol	2.5	2.5	2.5	2.5	2.5
Monocalcium phosphate	0.6	0.6	0.6	0.6	0.6
Titanium oxide	0.2	0.2	0.2	0.2	0.2

[1]Composition: 17.1% Ca; 2.7% P; 5.0% Mg; 0.1% K; 7.0% Na; 10.8% Cl; 723.5 ppm Cu; 2,584.2 ppm Fe; 5,694.4 ppm Zn; 41.9 ppm I; 17.2 ppm Co; 43.0 ppm Se; 927,500 IU Vit. A; 158,230 IU Vit. D; 5,250 IU Vit. E.

Table 2. Chemical composition of the pelleted calf starter mixtures differing in canola meal inclusion rate: 0, 15, 30, 45 and 60% of the CP originally supplied by soybean meal.

Variable	Treatment					Milk replacer
	0	15	30	45	60	
DM, %	96.4 ± 0.2[3]	96.2 ± 0.2	96.6 ± 0.3	96.5 ± 0.2	96.7 ± 0.3	94.3 ± 0.2
Ash, % DM	8.6 ± 0.3	7.6 ± 1.0	7.7 ± 0.9	8.8 ± 0.2	8.7 ± 0.9	6.6 ± 0.2
CP, % DM	24.4 ± 0.2	24.6 ± 0.4	24.2 ± 0.4	24.2 ± 0.3	24.1 ± 0.2	27.0 ± 0.4
ADF, % DM	7.2 ± 0.6	7.9 ± 0.3	8.3 ± 0.4	9.7 ± 0.4	9.9 ± 0.6	0.8 ± 0.5
NDF, % DM	17.4 ± 0.8	16.9 ± 0.6	18.4 ± 1.1	18.1 ± 0.3	18.2 ± 0.5	n/a
Starch, % DM	26.8 ± 0.9	26.8 ± 0.4	26.2 ± 0.4	26.6 ± 0.6	26.7 ± 0.4	n/a
WSC[1], % DM	13.6 ± 1.0	12.9 ± 1.0	13.6 ± 1.3	11.8 ± 2.0	12.3 ± 0.6	55.6 ± 6.5
EE[2], % DM	2.8 ± 0.1	2.5 ± 0.1	2.9 ± 0.1	2.7 ± 0.1	2.7 ± 0.2	20.2 ± 0.7
Ca, % DM	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.2	1.3 ± 0.1	1.4 ± 0.1	n/a
P, % DM	1.0 ± 0.0	0.9 ± 0.0	0.9 ± 0.1	1.0 ± 0.0	0.9 ± 0.0	n/a

[1]WSC = water soluble carbohydrates
[2]EE = ether extract
[3]Mean ± SD

Table 3. Apparent total tract digestibility post-weaning of pelleted calf starters differing in canola meal inclusion rate: 0, 15, 30, 45 and 60% of the CP originally supplied by soybean meal.

Variable	Treatment					SEM	*P*-value			
	0	15	30	45	60		Treatment	Linear	Quadratic	Cubic
Apparent total tract digestibility, %										
Dry matter	69.9	69.2	68.7	69.9	68.8	0.7	0.54	0.45	0.77	0.23
Crude protein	73.0	70.7	72.6	72.5	70.0	1.0	0.034	0.084	0.44	0.010
Starch	98.5	98.1	98.2	99.2	98.8	0.3	0.13	0.12	0.48	0.062
Ether extract	67.1	65.2	74.4	74.0	65.0	2.4	0.009	0.54	0.011	0.011

Table 4. Daily milk replacer (MR) and starter intake, and performance characteristics of calves fed different levels of canola meal inclusion: 0, 15, 30, 45 and 60% of the crude protein originally supplied by soybean meal.

Variable	Treatment					SEM	*P*-value			
	0	15	30	45	60		Treatment	Linear	Quadratic	Cubic
MR intake, kg DM/d										
Overall[1]	0.923	0.944	0.941	0.922	0.943	0.021	0.89	0.79	0.84	0.34
Milk-fed	1.034	1.073	1.052	1.051	1.055	0.028	0.86	0.80	0.59	0.41
Step down	0.796	0.789	0.813	0.765	0.822	0.030	0.46	0.70	0.53	0.32
Starter intake, kg DM/d										
Overall	0.997	0.998	0.987	0.938	0.839	0.063	0.30	0.055	0.30	0.84
Milk-fed	0.118	0.139	0.128	0.105	0.085	0.023	0.49	0.17	0.27	0.63
Step-down	0.893	0.934	0.903	0.866	0.666	0.091	0.21	0.063	0.14	0.74
Post-weaning	2.821	2.773	2.678	2.587	2.484	0.137	0.42	0.053	0.84	0.94
ADG, kg/d										
Overall	0.881	0.914	0.885	0.857	0.860	0.023	0.26	0.12	0.42	0.13
Milk fed	0.768	0.823	0.810	0.750	0.801	0.026	0.084	0.93	0.49	0.008
Step down	0.838	0.894	0.871	0.838	0.777	0.043	0.38	0.19	0.13	0.71
Post-weaning	1.212	1.164	1.096	1.159	1.104	0.060	0.49	0.18	0.55	0.55
Initial BW, kg	39.6	39.7	40.0	40.0	39.5	1.1	0.99	0.97	0.73	0.85
Final BW, kg	101.1	103.5	101.2	99.2	99.0	2.4	0.66	0.26	0.58	0.39
Cumulative growth, kg	61.5	63.8	61.2	59.2	59.5	2.2	0.59	0.22	0.69	0.30
Post-weaning feed efficiency, kg BW/kg feed	0.434	0.416	0.406	0.436	0.441	0.026	0.86	0.69	0.38	0.67

[1]Overall = d 8 to 71 of age; Milk-fed = d 8 to d 35 of age; Step-down = d 36 to 56 of age; Post-weaning = d 57 to 71 of age.

Table 5. Ruminal fermentation characteristics on d 70.2 ± 0.8 of age in calves fed pelleted starter mixtures differing in canola meal inclusion rate: 0, 15, 30, 45 and 60% of the crude protein originally supplied by soybean meal.

Variable	Treatment					SEM	*P*-value			
	0	15	30	45	60		Treatment	Linear	Quadratic	Cubic
Ruminal pH	5.65	5.54	5.70	5.51	5.57	0.15	0.84	0.64	0.97	0.98
SCFA[1] concentration, mM	118.3	133.1	111.3	132.6	128.0	6.7	0.083	0.33	0.85	0.62
Acetate, mol/100 mol	47.6	47.1	46.5	48.9	43.8	1.1	0.027	0.069	0.12	0.044
Propionate, mol/100 mol	36.0	38.0	36.0	38.3	40.9	2.5	0.27	0.075	0.39	0.45
Iso-butyrate, mol/100 mol	0.324	0.222	0.309	0.404	0.313	0.095	0.46	0.43	0.90	0.085
Butyrate, mol/100 mol	8.54	10.18	8.43	5.81	9.41	1.44	0.17	0.48	0.48	0.021
Iso-valerate, mol/100 mol	0.322	0.343	0.342	0.434	0.360	0.092	0.46	0.24	0.58	0.32
Valerate, mol/100 mol	3.73	4.49	3.45	3.50	3.23	0.43	0.042	0.034	0.36	0.12
Caprate, mol/100 mol	1.30	0.73	1.19	1.15	0.83	0.23	0.14	0.35	0.98	0.032
Ammonia, mg/dL	4.00	3.00	3.40	5.05	3.42	0.71	0.18	0.63	0.99	0.025

[1]SCFA = short-chain fatty acid.

Table 6. Blood parameters of calves on 62.2 ± 0.8 d of age as influenced by different canola meal inclusion rates in pelleted starter mixtures: 0, 15, 30, 45 and 60% of crude protein originally supplied by soybean meal.

Variable	Treatment					SEM	*P*-value			
	0	15	30	45	60		Treatment	Linear	Quadratic	Cubic
Glucose, mg/dL	62.7	61.1	61.8	58.8	61.7	2.0	0.71	0.50	0.46	0.56
Insulin, µg/L	0.549	0.559	0.433	0.403	0.594	0.066	0.11	0.73	0.045	0.061
BHBA[1], mmol/L	0.218	0.251	0.230	0.187	0.236	0.054	0.43	0.72	0.880	0.067
Urea, mg/dL	13.2	12.5	11.9	12.2	12.0	0.5	0.14	0.044	0.15	0.59

[1]BHBA = β-hydroxybutric acid.

Table 7. Time response of the blood parameters of calves on 62.2 ± 0.8 d of age as influenced by different canola meal inclusion rates in pelleted starter mixtures: 0, 15, 30, 45 and 60 of crude protein originally supplied by soybean meal.

Variable	Hour						SEM	*P*-value
	0200h	0600h	1000h	1400h	1800h	2200h		
Glucose, mg/dL	64.0[a]	62.3[ab]	60.3[bc]	61.5[b]	60.8[b]	58.3[c]	1.0	<0.001
Insulin, µg/L	0.703[a]	0.438[b]	0.531[ab]	0.509[ab]	0.502[ab]	0.542[ab]	0.0675	0.023
BHBA[1], mmol/L	0.222[a]	0.186[b]	0.216[ab]	0.222[a]	0.228[a]	0.238[a]	0.035	<0.001
Urea, mg/dL	12.3	12.5	12.4	12.3	12.4	12.3	0.4	0.35

[a,b,c] Means with uncommon superscripts differ ($P < 0.05$).

[1]BHBA = β-hydroxybutric acid.