Supplementing sodium butyrate to limit-fed heifers: 
Effects on growth, coccidiosis, urinary purine derivatives, 
and apparent total-tract nutrient digestibility

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ABSTRACT

The objective of this study was to assess the growth, apparent total-tract digestibility of nutrients, the prevalence of coccidia, and purine derivatives in postweaning heifers when limit-fed a diet supplemented with sodium butyrate (SB). A 12 wk randomized complete block experiment was conducted using 24 Holstein heifers (92.8 d ± 1.9 d of age and initial body weight [BW] of 99.6 ± 15.2 kg [mean ± standard deviation]). Treatments were 100 g soybean meal (control; CON) and 0.75 g of SB/kg of BW + 100 g soybean meal (SB). Diets were formulated to contain 16.4% crude protein, 2.27 Mcal/kg metabolizable energy (ME), and fed at a feed out rate of 2.15% of BW on a dry matter basis. Intakes were recorded daily while growth measurements and BW were recorded weekly. Urine and fecal samples were taken every 2 wk. On d 42 through d 49 an apparent total-tract digestibility phase took place using acid detergent insoluble ash as a marker. Growth measurements were similar among treatments except CON heifers grew longer and tended to be taller at the withers. A trend was observed for CON animals to have lower levels of coccidian oocytes by week. Heifers fed SB had lower blood glucose levels and higher levels of ketones in their blood. Urinary volume was greater for heifers fed SB throughout the 12 wk study. Total purine derivatives were greater in CON heifers. Dry matter, organic matter and acid detergent fiber digestibilities were greater for heifers fed SB compared with CON heifers. Crude protein, neutral detergent fiber, and ash digestibilities tended to be greater in heifers fed SB than in CON heifers. These results suggested no growth benefit of supplementing SB to limit-fed heifers; however, apparent total-tract fiber, ash, and crude protein digestibilities were improved in the SB fed heifers likely due to improved ruminal and intestinal development.

Key words: feed additive, precision-fed heifer, health, performance

INTRODUCTION

One of the largest expenses on a dairy farm is raising replacement heifers. Feed, reproduction, and health related costs including treating for disease are all costly expenses, with feed costs being the greatest expense. Cutting costs associated with heifers is challenging without compromising the quality of animals raised. A balance must be found to reduce costs and still ensure animals will reach maturity at a younger age. One management technique that can reduce costs is precision or limit feeding. This feeding style can decrease feed costs due to a lower quantity of feed being offered. In a limit-fed diet, the ration is more nutrient dense, therefore feed is offered at a percentage of BW, and nutrient requirements are met more precisely. Animals can use more of the nutrients included in the diet, increasing feed efficiency (FE) which in turn reduces feed costs.

While having a different mode of action, sodium butyrate (SB) has been shown to have similar benefits to ionophores in regard to FE and coccidiosis (Rice et al., 2019; Stahl et al., 2020). The FE responses have been seen through a more developed rumen, leading to an increase in papillae length and volume when calves are supplemented with SB in a starter grain (Górka et al., 2011a,b). This allows for the absorptive capacity to be increased within the rumen, utilizing more nutrients in feed (Górka et al., 2011a,b). Within the gastrointestinal tract, there has been shown to be an enhancement in the maturation of the small intestine epithelium when SB is included in milk replacer (Guilloteau et al., 2009; Górka et al., 2014). Sodium butyrate can also decrease cell apoptosis and increase cell proliferation in the small intestine (Górka et al., 2014). Pancreatic secretion is also shown to be increased when SB is fed, which aids in digestion (Guilloteau et al., 2010).

Postweaning heifers have also been shown to have positive effects when supplemented with SB such as an increase in BW, a tendency for greater FE and final
BW, and a reduction of coccidia oocysts when SB was included in heifer diets in different concentrations (Rice et al., 2019). Stahl et al. (2020), showed that SB was similar to monensin in heifer performance. Heifers were supplemented with SB, monensin, or a combination of both. When fed an additive, animals tended to have greater average BW, increased DMI, and a reduction in the prevalence of coccidia oocysts compared with the control animals (Stahl et al., 2020). Klobucher et al. (2022) indicated that SB and butyric acid (the dissociated acid component of SB) was effective in killing coccidial sporozoites in cell-culture.

The objective of this study was to evaluate SB supplementation to limit-fed postweaning heifers on growth performance, health, and urinary purine derivatives (PD). The hypothesis of this experiment was that SB in a limit-fed heifer diet will increase growth and apparent total-tract digestibility, and decrease coccidia.

**MATERIALS AND METHODS**

**Experimental Design and Treatments**

This experiment was reviewed and approved by the University of New Hampshire Animal Care and Use Committee (Protocol No. 210201).

Twenty-four Holstein heifers with a mean age of 92.8 d ± 1.9 d (mean ± SD) and average initial BW of 99.6 kg ± 15.2 kg (mean ± SD) were blocked by date of birth and randomly assigned to 1 of 2 treatments in a randomized complete block design. A power test was performed to determine correct sample size using results from ketone data from Rice et al. (2019). A sample size of n = 10 was determined to detect significance. Treatments were (1) carrier, 100 g soybean meal (control; CON); and (2) 0.75 g of SB/kg of BW + 100 g soybean meal (SB). All treatments were adjusted weekly according to individual BW, excluding control animals who received 100 g of soybean meal daily throughout the study. The SB provided was unprotected and was a 90% SB product with 68–69% butyric acid and approximately 21% to 22% Na+ and 10% maltodextrin (Ultramix GF, Adisseo Inc. USA, Alpharetta, GA). Heifers entered the pen to train to use Calan doors (American Calan Inc.) at 12 wk of life. The study began on the first Tuesday of the 13 wk of age. Heifers stayed on the experiment for 12 wk.

**Management and Feeding**

Heifers were group-housed in a naturally ventilated freestall barn with mattresses with no bedding provided. Three adjacent pens (pen 1: 5.46 × 4.75 m; pen 2: 5.54 × 4.88 m; pen 3: 6.32 × 4.8 m) were used. Pen 1 having the capacity to hold 6 heifers, pen 2 having the capacity to hold 8 heifers, and pen 3 having the capacity to hold 8 heifers. Automatically refilling water troughs allowed for free access to water throughout the study. There was no competition for stall space. Each heifer was given a training period that lasted an average of 9 d to train to the Calan doors (American Calan Inc., Northwood, NH).

Heifers on the current study were limit-fed compared with the conventional way of feeding heifers targeting at 10% orts and fed a TMR (Table 1) at approximately 0700 h daily in individual feed tubs to allow for daily feed intake measurements. Feed was mixed and distributed using a motorized feeding vehicle (Super Data Ranger; American Calan Inc.). The ration was fed to allow for consumption of 2.15% of BW on a DM basis. Prepubertal heifers are suggested to have 2.15% BW of DMI/d (Heinrichs and Zanton, 2016). The diet was formulated to have ME = 2.27 Mcal/kg and CP = 16.4%. The amount fed was adjusted weekly according to individual BW. Body weight measurements were taken every Monday at 1300 h and amounts fed were adjusted for the following morning feeding. Treatments were top dressed and hand-mixed into each individual feed tub. If refusals were present, it was recorded, and a sample was taken.

**Feed Analysis**

Feed offered to each heifer was measured daily at the time of feeding to determine DMI. Samples of TMR were collected daily and composited by week. When orts were present, a sample was collected and measured to determine DMI. Both TMR and refusal samples were frozen at −20°C for future analysis. Samples were thawed overnight and placed in a forced hot air convection oven (Binder, Bohemia, NY) to dry at 55°C for 48 h for determination of DM.

**Table 1. Ingredient composition (% of DM ± SD) of experimental diet**

<table>
<thead>
<tr>
<th>Item</th>
<th>DM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn silage</td>
<td>46.17 ± 1.75</td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>35.44 ± 2.90</td>
</tr>
<tr>
<td>Energy mix&lt;sup&gt;1&lt;/sup&gt;</td>
<td>3.14 ± 0.94</td>
</tr>
<tr>
<td>Soy-urea mix&lt;sup&gt;2&lt;/sup&gt;</td>
<td>12.19 ± 3.55</td>
</tr>
<tr>
<td>Mineral-vitamin mix&lt;sup&gt;3&lt;/sup&gt;</td>
<td>3.06 ± 0.03</td>
</tr>
</tbody>
</table>

<sup>1</sup>Energy mix contained 5% molasses, 45.80% corn meal, 15.20% steam-flaked corn, and 34% whole beet pulp.

<sup>2</sup>Soy-urea mix contained 7.28% distillers grain, 69.14% soybean meal, 21.83% canola meal, 1.75% urea.

<sup>3</sup>Mineral-vitamin mix contained 17.28% Ca, 6.01% P, 3.0% Mg, 23.70% salt, 7.80% Na, 0.29% Fe, 0.26% Zn, 0.26% Mn, 12.3% Cl, 602.00 mg/kg Cu, 15.00 mg/kg Co, 20.00 mg/kg Se, 15.00 mg/kg I, 267,800 IU/kg vitamin A, 111,071 IU/kg vitamin D, and 2,207 IU/kg vitamin E.
Dried samples were ground through a 1-mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ) and sent to a laboratory for nutrient analysis (Dairy One Forage Laboratory, Ithaca, NY). Feed samples were analyzed for the following: Acid detergent fiber (method 5 in an Ankom Fiber Analyzer A2000; Ankom Technology; method 973.18, AOAC International, 1998), neutral detergent fiber (method 6 in an Ankom Fiber Analyzer A2000 with amylase and sodium sulfite; Ankom Technology, Fairpoint, NY; solutions as in Van Soest et al., 1991), starch (YSI 2700 SELECT Biochemistry Analyzer; YSI Inc. Life Sciences, Yellow Springs, OH), crude fat (ether extraction; AOAC 2003.05; AOAC International, 2006), ash (AOAC Method 942.05; AOAC International, 2006), and CP (AOAC method 990.03; AOAC International, 2006).

**Measurements and Blood Sampling Analysis**

Body weight and skeletal measurements were taken weekly at 1330 h every Monday throughout the study. Heifers were measured for body length, heart girth, and paunch girth using a weigh tape. Animals were also measured for withers height and hip height using a sliding scale height stick with bubble level. Heifers were weighed on a portable scale (Tru-TestTM EziWeigh5i, Uniontown, PA).

Blood samples were obtained from the jugular vein using a 20-gauge needle before the time of measurements for the duration of the study. Samples were collected in two 10-mL Vacutainer tubes, one containing anticoagulant EDTA and the other without an anticoagulant (Monoject, Covidien LLC, Mansfield, MA). Blood ketone concentrations were obtained using a hand-held electronic blood glucose and ketone monitoring device (Nova Max Plus, Nova Biomedical, Waltham, MA; Deelen et al., 2016). Ketone concentrations were determined by taking a whole-blood sample, not containing EDTA, and transferring it to the sensor of a test strip using a disposable pipette. Blood ketone levels were run in duplicate. Samples with EDTA were placed on ice until they were centrifuged at 1,278 × g at 4°C for 20 min (5430R, Eppendorf, Hamburg, Germany). Plasma was frozen at −20°C until further analysis. Plasma was thawed and plasma glucose concentrations were measured in duplicate via Wako Autokit for Glucose (Wako Diagnostics, Mountain View, CA) and read on a UV-visible spectrophotometer at a wavelength of 505 nm.

**Digestibility Measurements**

Each of the 24 heifers underwent apparent total-tract nutrient digestibility phase on d 42 on the study until d 49. Total mixed ration samples were taken Tuesday through Saturday and composited over 5 d. Total mixed ration samples were then frozen at −20°C for future analysis. Samples were thawed and placed in a forced hot air convection oven to dry at 55°C for 48 h to determine DM. Fecal grab samples were collected on Friday, Saturday, Sunday, and Monday every 12 h to represent a 24-h period (d 4: 0200 and 1400 h; d 5: 0500 and 1700 h; d 6: 0800 and 2000 h; d 7: 1100 and 2300 h) by stimulating defecation or collecting feces directly from the rectum. Fecal samples over the 4-d period were combined to obtain a single composite and frozen at −20°C. Fecal samples were thawed at room temperature and emptied into aluminum trays to be dried in a forced-air oven at 55°C for 72 h until dried. The dried TMR and fecal samples were ground through a 1-mm screen Wiley mill (Thomas Scientific, Swedesboro, NJ). Ground samples were sent to Dairy One Forage Laboratory (Ithaca, NY) for analysis. Feed and fecal samples were analyzed for acid detergent insoluble ash (ADIA) according to Van Keulen and Young (1977), and CP, NDF, ADF, starch, ash, and fat as previously described.

The equation used to estimate digestibility was 100 − [100 × (% ADIA in DM consumed/% ADIA in feces) × (% nutrient in feces/nutrient consumed DM)] (Maynard et al., 1979).

**Coccidia Enumeration**

Fecal samples were obtained from each heifer before the start of treatment and then every 2 wk for the entire study. Samples were taken by stimulation or directly from the rectum from each heifer on Monday at 1330 h and analyzed for coccidian oocysts following the modified Wisconsin sugar fecal worm egg flotation method (Bliss and Kvasnicka, 1997). Heifers were observed daily for scour.

**Urine Analysis**

Urine samples were collected from each heifer before the start of treatment and every 2 wk thereafter (1300 h) through direct stimulation of the pudendal nerve. Samples were immediately brought to the laboratory where 8.4 mL of the sample was deposited into a centrifuge tube containing 32 mL of 0.072 N H₂SO₄ and frozen for later analysis.

Samples were thawed at room temperature before analysis for creatinine, allantoin, and uric acid. Samples were analyzed colorimetrically for creatinine (assay kit #500701, Cayman Chemical Co., Ann Arbor, MI) and uric acid (Quantichrom Uric Acid Kit DIUA-250, Hayward, CA) using a microplate reader (Epoch Bio Tek Instruments Inc., Winooski, VT) set at a wave-
Statistical Analysis

Initial measurements of BW, skeletal measurements, glucose, ketones, PD, and coccidia counts were used as covariates for their respective variable. Weekly measurements of DMI, ADG, FE (ADG/DMI), BW, skeletal measurements, coccidia counts, glucose, ketones, and PD were analyzed as a randomized complete block design with repeated measures using the MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC) according to the following model:

\[ Y_{ijkl} = \mu + B_i + Trt_j + W_k + \beta X_{ij} + TrtW_{jk} + E_{ijkl}, \]

where \( Y_{ijkl} \) = the dependent variable; \( \mu \) = the overall mean; \( B_i \) = the random effect of block \( i (i = 1, \ldots, 13) \); \( Trt_j \) = the fixed effect of the \( j \)th treatment \( (j = \text{control, SB}) \); \( W_k \) = the fixed effect of the \( k \)th week on study \( (k = 1–12) \); \( \beta \) = the regression (covariate coefficient); \( X_{ij} \) = the covariate measurement; and \( E_{ijkl} \) = the residual error ~N(0, \( \sigma^2_e \)).

Final and initial measurements, overall gains, and apparent tract-total digestibility were analyzed using the MIXED procedure of SAS 9.4 (SAS Institute Inc.) according to the following model:

\[ Y_{ijk} = \mu + B_i + Trt_j + E_{ijk}, \]

where \( Y_{ijk} \) = the dependent variable; \( \mu \) = the overall mean; \( B_i \) = the random effect of block \( i (i = 1, \ldots, 13) \); \( Trt_j \) = the fixed effect of the \( j \)th treatment \( (j = \text{control, SB}) \); \( E_{ijk} \) = the residual error ~N(0, \( \sigma^2_e \)).

Significant treatment effects were determined significant when \( P < 0.05 \) and trends at \( 0.05 \leq P \leq 0.10 \). All data points greater or lesser than 2.5 SD away from the mean were considered outliers and removed. The Univariate procedure of SAS 9.4 (SAS Institute Inc.) was used to evaluate normal distribution of data. If data were not normally distributed, data transformation was conducted to allow for normal distribution before conducting statistical analysis. If data were not able to be transformed, the Glimmix procedure (SAS Institute Inc.) was used on the nontransformed data.

RESULTS

Nutrient analysis of the TMR throughout the 9-mo trial is shown in Table 2. Intake and performance as described by skeletal measurements, DMI, FE, ADG, and BW are presented in Table 3. Two heifers were treated for coccidiosis according to farm protocols with Amprilium (Corid, Huvepharma, Peachtree City, GA) as a drench. Both animals were on the SB treatment. Orts were only seen in these heifers throughout the study when they were being treated for coccidiosis. One heifer from the SB treatment was removed from the study due to severe coccidiosis and Salmonella infection. One heifer was added to account for the animal removed from the study. This caused 2 incomplete blocks in the study. Data from the heifer that was removed were not used in the experiment. Each treatment had 12 heifers complete the study.
There were no differences in average BW, ADG, initial BW, and final BW across the 2 treatments. Average BW ($P = 0.04$) and ADG ($P < 0.01$) was significant by week. Withers height tended to be greater ($P = 0.06$) and body length gain were greater ($P = 0.03$) for CON treatment compared with SB treatment. No other skeletal measurement differences were observed. Overall gains are reported in Table 3 and no difference was observed between treatments.

Coccidia and blood parameters results are presented in Table 4. No difference was observed between treatments for initial coccidia counts per kilogram of feces, coccidia counts per kilogram of feces, and coccidia rate of incidence. Rate of incidence was determined by if any oocysts were present in the feces. Coccidia counts per kilogram of feces tended to be greater ($P < 0.10$) by week for the SB treatment. Blood glucose concentrations tended to be greater ($P < 0.10$) for animals on the CON diet. Final glucose concentrations tended to be greater ($P = 0.09$) for CON heifers. Average ketone concentrations were greater ($P < 0.0001$) in heifers on the SB treatment when compared with the CON. Final ketone concentrations were also greater ($P < 0.01$) for animals on the SB treatment compared with heifers on the CON treatment.

Purine derivative excretion and urine volumes are shown in Table 5. Heifers supplemented with SB had a greater urine volume per day ($P = 0.01$) compared with the CON heifers. Allantoin production was greater ($P < 0.01$) in heifers fed the CON treatment compared with heifers fed SB. Uric acid production was similar between treatments. Total PD yield was greater ($P = 0.02$) for CON heifers compared with SB supplemented heifers.

The results from the apparent total-tract digestibility are presented in Table 6. Animals supplemented with SB had greater digestibility of DM ($P = 0.05$), ADF ($P = 0.04$) and OM ($P = 0.04$). There was a tendency for digestibility to increase when heifers were supplemented with SB for the following nutrients: CP ($P = 0.07$), NDF ($P = 0.06$), and ash ($P = 0.10$). Purine derivatives for wk 6 are presented and were similar between treatments except for a trend for uric acid yield to be greater ($P = 0.09$).

### DISCUSSION

Previous research in supplementing SB to postweaning heifer diets contradict the present study. Rice et al. (2019), observed an increase in BW, and a tendency for greater FE and final BW when heifers were supplemented with SB had greater digestibility of DM ($P = 0.05$), ADF ($P = 0.04$) and OM ($P = 0.04$). There was a tendency for digestibility to increase when heifers were supplemented with SB for the following nutrients: CP ($P = 0.07$), NDF ($P = 0.06$), and ash ($P = 0.10$). Purine derivatives for wk 6 are presented and were similar between treatments except for a trend for uric acid yield to be greater ($P = 0.09$).

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**Table 2.** Nutrient analysis (% of DM ± SD) of experimental diet

<table>
<thead>
<tr>
<th>Item</th>
<th>DM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>16.92 ± 1.09</td>
</tr>
<tr>
<td>NDF</td>
<td>27.64 ± 2.14</td>
</tr>
<tr>
<td>ADF</td>
<td>38.95 ± 2.50</td>
</tr>
<tr>
<td>Ash</td>
<td>8.50 ± 0.53</td>
</tr>
<tr>
<td>Starch</td>
<td>17.30 ± 2.85</td>
</tr>
<tr>
<td>NFC1</td>
<td>31.53 ± 2.64</td>
</tr>
<tr>
<td>Fat</td>
<td>4.11 ± 0.62</td>
</tr>
<tr>
<td>ME2</td>
<td>2.27</td>
</tr>
</tbody>
</table>

1NFC = 100 − [CP% + (NDF% − NDICP%) + fat% + ash%]. NDICP = neutral detergent insoluble crude protein.

2Estimated from NRC (2001).

**Table 3.** Intake and performance of limit-fed heifers fed 0 g/kg sodium butyrate or 0.75 g/kg BW sodium butyrate from 12 to 24 wk of age

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment1</th>
<th>$P$-value2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>SB</td>
</tr>
<tr>
<td>Average BW, kg</td>
<td>135.10</td>
<td>134.50</td>
</tr>
<tr>
<td>Overall BW gain, kg</td>
<td>70.40</td>
<td>70.50</td>
</tr>
<tr>
<td>ADG, kg/d</td>
<td>0.83</td>
<td>0.86</td>
</tr>
<tr>
<td>Initial BW, kg</td>
<td>99.40</td>
<td>99.70</td>
</tr>
<tr>
<td>Final BW, kg</td>
<td>168.50</td>
<td>170.40</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>3.02</td>
<td>2.92</td>
</tr>
<tr>
<td>Feed efficiency, ADG/DWI</td>
<td>0.28</td>
<td>0.29</td>
</tr>
<tr>
<td>Heart girth, cm</td>
<td>114.70</td>
<td>113.40</td>
</tr>
<tr>
<td>Overall heart girth gain, cm</td>
<td>21.40</td>
<td>21.40</td>
</tr>
<tr>
<td>Paunch girth, cm</td>
<td>139.00</td>
<td>138.20</td>
</tr>
<tr>
<td>Overall paunch girth, cm</td>
<td>28.40</td>
<td>27.40</td>
</tr>
<tr>
<td>Withers height, cm</td>
<td>102.80</td>
<td>101.40</td>
</tr>
<tr>
<td>Overall withers height gain, cm</td>
<td>14.10</td>
<td>13.70</td>
</tr>
<tr>
<td>Hip height, cm</td>
<td>105.50</td>
<td>105.80</td>
</tr>
<tr>
<td>Overall hip height gain, cm</td>
<td>13.00</td>
<td>13.70</td>
</tr>
<tr>
<td>Length, cm</td>
<td>90.70</td>
<td>90.20</td>
</tr>
<tr>
<td>Overall length gain, cm</td>
<td>17.50</td>
<td>16.20</td>
</tr>
</tbody>
</table>

1Treatment: CON = 0 g/d SB; SB = 0.75 g sodium butyrate/kg BW.

2$P$-value significant if <0.05; trend if <0.10. TRT = treatment.
mented SB in increasing concentrations (0 to 0.75 g/kg). Stahl et al. (2020), also observed many benefits to supplementing additives, either supplementing SB (0.75 g/kg), MON (1 mg/kg), or the combination to heifer diets. These researchers found that when heifers were fed either additive, they tended to have a greater average BW and increased DMI.

Due to BW being included in the calculation for the amount fed to limit-fed heifers, DMI could only increase if BW increased. No effect of DMI was seen in the present study. These data are similar to others based on limitations to intake on limit-fed heifers (Hoffman et al., 2007). Hoffman et al. (2007), limited intake on heifers and saw a linear decrease in DMI due to the nature of the feeding approach used. Feed efficiency is expressed as ADG/DMI in the current study. With no difference seen in ADG or DMI, FE was the same across treatments. Rice et al. (2019) had a similar result, observing 0.28 for the 0.75 g SB/kg BW treatment in ad libitum fed heifers. However, Stahl et al. (2020) observed a FE result of 0.25 for the SB treatment in ad libitum fed heifers. This is supported by Pino et al. (2018), observing ad libitum fed animals to have a lesser FE response; therefore, limit-fed animals have improved FE. Average daily gain had a treatment by week interaction, due to the strong significance of week in the present study. This same result occurred for average BW. Final BW was the same across treatments. Rice et al. (2019) saw a linear trend for final BW to increase when supplementing SB; Stahl et al. (2020) also saw a trend for final BW to be greater when supplementing SB, MON, or the combination compared with no additive. Manthey et al. (2016) used the limit feeding strategy and observed no difference in growth of heifers as diet as a proportion of BW decreased but nutrient density was similar. Hoffman et al. (2007) observed no difference in growth between ad libitum fed heifers and limit-fed heifers. Supplementing SB resulted in a trend for daily withers height gain, and length gain to be reduced compared with CON heifers. Overall gains were the same among treatments.

Table 4. Coccidia count and rate, plasma glucose, and whole-blood ketones of heifers limit-fed 0 g/kg BW sodium butyrate or 0.75 g/kg BW sodium butyrate from 12 to 24 wk of age

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>SB</td>
</tr>
<tr>
<td>Initial coccidia/kg of feces</td>
<td>16.60</td>
<td>15.40</td>
</tr>
<tr>
<td>Coccidia/kg of feces</td>
<td>8,616.50</td>
<td>10,815.00</td>
</tr>
<tr>
<td>Coccidia rate, %</td>
<td>0.65</td>
<td>0.56</td>
</tr>
<tr>
<td>Initial glucose, mg/dL</td>
<td>90.90</td>
<td>91.90</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>95.00</td>
<td>90.42</td>
</tr>
<tr>
<td>Final glucose, mg/dL</td>
<td>107.00</td>
<td>92.99</td>
</tr>
<tr>
<td>Initial ketones, mmol/L</td>
<td>0.78</td>
<td>1.20</td>
</tr>
<tr>
<td>Ketones, mmol/L</td>
<td>0.93</td>
<td>1.55</td>
</tr>
<tr>
<td>Final ketones, mmol/L</td>
<td>0.97</td>
<td>1.31</td>
</tr>
</tbody>
</table>

1Treatment CON = 0 g/d SB; SB = 0.75 g sodium butyrate/kg BW.
2P-value significant if <0.05; trend if <0.10. TRT = treatment.
3Percent of weeks when coccidia were present in any heifer.

Table 5. Purine derivative excretion of heifers limit-fed 0 g/kg BW sodium butyrate or 0.75 g/kg BW sodium butyrate from 12 to 24 wk of age

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>SB</td>
</tr>
<tr>
<td>Urinary volume, L/d</td>
<td>7.00</td>
<td>9.23</td>
</tr>
<tr>
<td>Allantoin, mmol/d</td>
<td>245.40</td>
<td>104.80</td>
</tr>
<tr>
<td>Uric acid, mmol/d</td>
<td>16.60</td>
<td>14.40</td>
</tr>
<tr>
<td>Total PD, mmol/d</td>
<td>257.00</td>
<td>120.00</td>
</tr>
</tbody>
</table>

1Treatment CON = 0 g/d SB; SB = 0.75 g sodium butyrate/kg BW.
2P-value significant if <0.05; trend if <0.10. TRT = treatment.
3Data were transformed to 1/square root and then transformed back. The SEM was not transformed back.
4Data were transformed to the square root for normal distribution and then transformed back. The SEM was not transformed back.
5PD = purine derivatives.
Table 6. Apparent total-tract nutrient digestibility (%) and urinary purine derivatives during week 6

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>SEM</th>
<th>P-value (TRT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item</td>
<td>Control</td>
<td>SB</td>
<td>SEM</td>
</tr>
<tr>
<td>DMI</td>
<td>2.94</td>
<td>2.91</td>
<td>0.03</td>
</tr>
<tr>
<td>DM</td>
<td>63.80</td>
<td>66.90</td>
<td>1.00</td>
</tr>
<tr>
<td>CP</td>
<td>63.80</td>
<td>67.10</td>
<td>1.20</td>
</tr>
<tr>
<td>ADF</td>
<td>45.20</td>
<td>50.50</td>
<td>1.60</td>
</tr>
<tr>
<td>NDF</td>
<td>50.70</td>
<td>55.00</td>
<td>1.40</td>
</tr>
<tr>
<td>HCell(^{1,4})</td>
<td>61.90</td>
<td>66.40</td>
<td>0.20</td>
</tr>
<tr>
<td>Ash</td>
<td>48.40</td>
<td>52.80</td>
<td>1.80</td>
</tr>
<tr>
<td>Starch</td>
<td>97.60</td>
<td>97.70</td>
<td>0.20</td>
</tr>
<tr>
<td>Fat</td>
<td>75.90</td>
<td>79.20</td>
<td>2.20</td>
</tr>
<tr>
<td>OM</td>
<td>66.70</td>
<td>71.20</td>
<td>1.40</td>
</tr>
<tr>
<td>Allantoin(^{4})</td>
<td>126.90</td>
<td>69.50</td>
<td>1.23</td>
</tr>
<tr>
<td>Uric acid, mmol/d</td>
<td>17.80</td>
<td>13.50</td>
<td>1.58</td>
</tr>
<tr>
<td>Total PD(^{1})</td>
<td>137.30</td>
<td>91.10</td>
<td>21.30</td>
</tr>
<tr>
<td>Urine volume, L/d</td>
<td>10.20</td>
<td>9.83</td>
<td>2.01</td>
</tr>
</tbody>
</table>

\(^{1}\)Treatment CON = 0 g/d SB; SB = 0.75 g sodium butyrate/kg BW.

\(^{2}\)P-value significant if <0.05; trend if <0.10. TRT = treatment.

\(^{3}\)Hemicellulose = NDF − ADF.

\(^{4}\)Data were converted to square root to allow for normal distribution and then converted back. The SEM was not converted back.

\(^{5}\)PD = purine derivatives.

Rate of passage of nutrients through the digestive tract slows for animals who are limit-fed (Zanton and Heinrichs, 2008; Pino et al., 2018). These researchers showed that retention time in the rumen increases with limit-fed diet and decreases in ad libitum diets. Based on the higher concentration of ketones in the blood observed in this study, it is assumed that SB was dissociating in the rumen. Ketone concentrations were 50% less in the SB ad libitum fed heifer studies (0.61 mmol/L, Rice et al., 2019; 0.50 mmol/L, Stahl et al., 2020) than those in this experiment (0.93 mmol/L CON; 1.55 mmol/L SB). Sodium ions are ionically bound to butyrate, and in the rumen this bond is broken, allowing for the epithelium to convert the butyrate molecule to ketone bodies (Holtenius and Holtenius, 1996; Müller et al., 2002; Herrick et al., 2017; Rice et al., 2019; Stahl et al., 2020). During the phase from preruminant to ruminant there is a change from glucose absorption in the intestines to gluconeogenesis in the liver (Baldwin et al., 2004). Sodium butyrate increases hepatic enzymes resulting in less carbohydrate available for postruminal digestion leading to a decrease in glucose absorption (Rice et al., 2019; Stahl et al., 2020). These researchers saw a reduction in plasma glucose which is consistent with the present study. It is also consistent with lactating cow studies when butyrate or SB was infused (Huhtanen et al., 1993; Herrick et al., 2017).

No effect of treatment was seen in the present study on coccidial oocysts. This contradicts other studies when SB was supplemented to heifers (Rice et al., 2019; Stahl et al., 2020). Possibly, due to the assumed decreased rate of passage in heifers of the current study, no element of the SB molecule may have reached the small intestine to reduce inflammation, heal intestinal cells, or cause merozoite destruction as seen in the other studies.

Purine derivatives have a direct relationship to microbial protein synthesis and is a noninvasive way to estimate microbial CP (Pina et al., 2009). To our knowledge, this is the first study to evaluate limit-fed heifers supplemented with SB on PD production. The lower amount PD in the SB supplemented heifers may be due an alteration in the microbial community as seen with broilers supplemented with SB (Wu et al., 2018). These researchers fed growing broilers at a rate similar to the present experiment (800 mg/kg BW) and observed increases in Bacteroidetes and Ruminococcaceae and a large decrease in Enterobacteriaceae. The PD method does not allow for bacterial species differentiation. Alternatively, the reduced passage rate in limit-fed heifers, could have resulted in a lower rumen pH due to a reduced passage of SB out of the rumen. The lower pH could have reduced microbial synthesis.

Urine volume was increased in heifers fed SB. The SB used in this study was 21% sodium, therefore increasing the sodium consumed by animals on the SB treatment. During the final week of the trial, heifers fed SB consumed on average 121.7 g/d SB therefore consuming 25.6 g/d more sodium than CON heifers. Using the equation from Murphy et al. (1983), heifers fed the SB treatment would be consuming on average 1.28 L/d more water than the CON heifers. This increase in water consumption would lead to an increase in urine volume. Lee et al. (2019) suggested that more spot samples should be taken over a day to reduce diurnal variations in creatinine in lactating cows resulting in differing urine volumes. In this study, heifers were used and samples were taken at the same time each day every 2 weeks. Results may have been different if more samples were taken on the sampling day as suggested by Lee et al. (2019). These researchers observed that urine output was the same between spot sampling at 10 h after feeding and total collection. Lee et al. (2019) attributed the lack of statistical significance to greater SEM for creatinine and urine output than for those with multiple spot samples. These same researchers stated that spot urine sampling can be conducted to evaluate differences in diets fed to cows. Likely, a similar outcome would occur with heifers, but research in this area is lacking.

Apparent total-tract digestibility was increased for DM, ADF and OM for animals on the SB treatment. There was a tendency for SB to increase digestibility of CP, NDF, and ash. With the dissociating of SB in the
rumen, butyrate is used by the rumen epithelium to increase growth and concentration of ruminal papillae (Rice et al., 2019; Stahl et al., 2020). This would result in an increase in the surface area of the rumen. The heifer is then more efficient at absorbing nutrients from fermentation. The results of ketone and plasma glucose data seen here support the hypothesis of the limit-fed SB supplemented animals having a more developed rumen. This part of the study was conducted during wk 6 of the experiment and there were no differences in urinary PD at this time suggesting that the reduced PD observed over the study likely occurred during the subsequent 6 wk and that SB did not reduce microbial protein synthesis during the digestibility phase of the experiment.

CONCLUSIONS

Overall, the supplementation of SB to limit-fed heifers did not affect growth parameters. Blood ketones and glucose levels were affected indicating development of the rumen due to SB. Coccidia counts were not reduced by SB, likely due to the SB being absorbed in the forestomach. Urinary volume was increased in animals supplemented with SB. Purine derivatives were reduced over the entire study for heifers fed SB compared with the CON heifers indicating that SB reduced microbial protein synthesis likely due to the increased residence time in the rumen of SB reducing rumen pH or a modification in bacterial species. There were no growth benefits of supplementing SB to limit-fed postweaning heifers, but fiber and CP digestibilities were improved. More research needs to be conducted to determine the optimum dose of SB to feed limit-fed heifers.

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