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# Abomasal infusion of corn starch and β-hydroxybutyrate in early-lactation Holstein-Friesian dairy cows to induce hindgut and metabolic acidosis

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## ABSTRACT

The objectives of this study were to induce hindgut and metabolic acidosis via abomasal infusion of corn starch and β-hydroxybutyrate (BHB), respectively, and to determine the effects of these physiological states in early-lactation dairy cows. In a 6 × 6 Latin square design, 6 rumen-fistulated Holstein-Friesian dairy cows (66 ± 18 d in milk) were subjected to 5 d of continuous abomasal infusion treatments followed by 2 d of rest. The abomasal infusion treatments followed a 3 × 2 factorial design, with 3 levels of corn starch and 2 levels of BHB. The infusions were water as control, 1.5 kg of corn starch/d, 3.0 kg of corn starch/d, 8.0 mol BHB/d, 1.5 kg of corn starch/d + 8.0 mol BHB/d, or 3.0 kg of corn starch/d + 8.0 mol BHB/d. A total mixed ration consisting of 35.0% grass silage, 37.4% corn silage, and 27.6% concentrate (on a dry matter basis) was fed at 90% of ad libitum intake of individual cows. The experiment was conducted in climate respiration chambers to facilitate determination of energy and N balance. Fecal pH decreased with each level of corn starch infused into the abomasum and was 6.49, 6.00, and 5.15 with 0.0, 1.5, and 3.0 kg of corn starch/d, respectively, suggesting that hindgut acidosis was induced with corn starch infusion. No systemic inflammatory response was observed and the permeability of the intestine or hindgut epithelium was not affected by the more acidic conditions. This induced hindgut acidosis was associated with decreased digestibility of nutrients, except for crude fat and NDF, which were not affected. Induced hindgut acidosis did not affect milk production and composition and energy balance, but increased milk N efficiency. Abomasal infusion of BHB resulted in a compensated metabolic acidosis, which was characterized by a clear disturbance of acid-base status (i.e., decreased blood total $CO_2$, $HCO_3$, and base excess, and a tendency for decreased urinary pH), whereas blood pH remained within a physiologically normal range. Abomasal infusion of BHB resulted in increased concentrations of BHB in milk and plasma, but both remained well below the critical threshold values for subclinical ketosis. Induced compensated metabolic acidosis, as a result of abomasally infused BHB, increased energy retained as body fat, did not affect milk production and composition or inflammatory response, but increased intestinal permeability.
**Key words:** dairy cow, early lactation, metabolic acidosis, hindgut acidosis

## INTRODUCTION

In early lactation, the rapid increase in energy requirements for milk production by dairy cattle cannot be met by an increase in feed intake alone (van Knegsel et al., 2007a). To compensate for this energy deficit, dairy cows mobilize body fat and, as a result, the concentration of nonesterified fatty acids (**NEFA**) is elevated in blood. When the capacity of the liver to oxidize these NEFA is exceeded, remaining NEFA are stored as triglycerides in the liver or are converted into ketone bodies that enter circulation (Bell, 1995). Although ketone bodies can be used as an alternative form of energy by some tissues (e.g., brain; Laffel, 1999), the utilization of ketone bodies is limited. Excess concentration of ketone bodies in blood increases the risk for ketosis (Duffield, 2000). Subclinical ketosis in early lactation affects 40 to 60% of cows in herds undergoing repeated testing (Emery et al., 1964; Simensen et al., 1990), which is much higher than the typical 2 to 15% incidence of clinical ketosis (Duffield, 2000). The ketone bodies BHB and acetoacetate are organic acids (pKa of 3.6 and 4.7, respectively) that dissociate almost fully at physiological pH (Laffel, 1999). If the associated rise in hydrogen ion concentration in blood exceeds blood buffering capacity during periods of high blood ketone concentrations, metabolic acidosis may result (Hood and Tannen, 1994).

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It has only been relatively recently recognized that strategies to prevent diseases such as ketosis in early-lactation cows (i.e., increasing the energy density of rations with fermentable carbohydrates) are closely linked with the gastrointestinal function and health of dairy cows (e.g., Enemark, 2008; Mulligan and Doherty, 2008). Hindgut fermentation has been associated with high intake of starch and other fermentable substrates that are not fully degraded in the rumen (Gressley et al., 2011). As dairy cows have relatively limited amylolytic activity in the small intestines (Mathé et al., 2001), incompletely degraded and digested substrates flow into the hindgut where fermentation of these substrates occurs. This increases the risk of hindgut acidosis, which can be defined as an accumulation of organic acids and a subsequent decrease in digesta pH (Gressley et al., 2011). Acidosis in the hindgut can potentially cause dysbiosis in the microbial community and epithelial damage, and consequently can affect animal production and health (Plaizier et al., 2018).

Recently, van Gastelen et al. (2021) showed that both hindgut acidosis and metabolic acidosis were associated with changes in feed intake, apparent total-tract digestibility (**ATTD**) of nutrients, milk production and composition, and N and energy partitioning. Importantly, van Gastelen et al. (2021) demonstrated that metabolic acidosis became more severe when dairy cows also experienced hindgut acidosis, suggesting that a relation may exist between acidosis in the hindgut and the occurrence of metabolic acidosis. In the study of van Gastelen et al. (2021), metabolic acidosis was induced by infusing ammonium chloride and hindgut acidosis was induced by infusing ground corn. It is unclear whether the relation between hindgut acidosis and metabolic acidosis also exists when metabolic acidosis is induced via accumulation of ketone bodies (i.e., a different mechanism that can affect the acid-base status of a dairy cow). It also remains unclear from the study of van Gastelen et al. (2021) whether there is a dose effect of the amount of starch infused and the severity of hindgut acidosis. Therefore, the aims of the current study were to (1) induce hindgut and metabolic acidosis via abomasal infusion of corn starch and BHB, respectively, and (2) determine the effects of these physiological states on feed intake, ATTD of nutrients, energy and N partitioning, milk production and composition, acid-base status, respiratory and metabolic status, and ruminal and hindgut fermentation characteristics. We hypothesized that abomasally infused corn starch would be fermented in the hindgut, produce VFA, and subsequently decrease digesta pH, resulting in hindgut acidosis and compromised intestinal permeability. Abomasal infusion of BHB was expected to disturb the acid-base status of the dairy cows, resulting in metabolic acidosis (i.e., a decreased blood pH). When corn starch and BHB were infused simultaneously into the abomasum, we expected to induce hindgut and metabolic acidosis. We expected the metabolic acidosis to be more severe with both corn starch and BHB infusion compared with only BHB infusion because of a combined effect of both types of acidosis on the acid-base status of the cow.

## MATERIALS AND METHODS

### Experimental Design and Housing

The experiment was conducted from August until October 2019 at the animal research facilities of Wageningen University & Research (Wageningen, the Netherlands), under the Dutch Law on Animal Experiments in accordance with European Union Directive 2010/63, and was approved by the Central Committee of Animal Experiments (The Hague, the Netherlands; 2018.D-0013.002).

Six rumen-fistulated, multiparous Holstein-Friesian dairy cows (second parity n = 5, third parity n = 1) producing 28.8 ± 4.96 kg/d at 66 ± 18 DIM (average ± SD) at the beginning of the experiment were randomly assigned to a 6 × 6 Latin square design with 6 treatments. Each experimental period (n = 6) consisted of 5 d of continuous abomasal infusion followed by 2 d of rest. Cows were adapted to the experimental conditions for 19 d before the first experimental period. For the first 14 d of adaptation, cows were housed individually in tiestalls to become adapted to the basal diet as well as the restriction in movement. For the last 5 d of adaptation as well as for the 6 consecutive experimental periods, cows were housed individually in identical climate respiration chambers (**CRC**) to measure, among other variables, the effect of abomasally infused BHB and corn starch on the energy and N partitioning of the dairy cows.

### Diet and Feeding

Cows were fed a TMR consisting of 35.0% grass silage, 37.4% corn silage, and 27.6% concentrate on a DM basis (Table 1). To determine the ATTD of nutrients, $TiO_2$ was included in the concentrate (5 g/kg DM) as an external marker. The concentrate was in meal form and produced by Research Diet Services (RDS BV) in a single batch. The diet was formulated to meet 101 and 96% of $NE_L$ (Van Es, 1978) and intestinal digestible protein requirements (Van Duinkerken et al., 2011), respectively, for cows consuming 19.0 kg of DM/d and producing 33 kg/d of milk containing 4.5%

fat and 3.5% protein (i.e., mean DMI and milk production measured during the first weeks of lactation of the cows that would potentially enter this study).

Cows were fed ad libitum for the first 10 d of the 19-d adaptation period. Intake during the final 5 d of this 10-d ad libitum intake period was used to calculate a 10% daily intake restriction for individual cows. From d 11 onward, cows were fed this fixed amount for the remainder of the adaptation and experimental periods. The grass silage, corn silage, and water were mixed 3 times weekly using a self-propelled mixer wagon (Strautmann Verti-Mix 500) equipped with a cutter loader system and an electronic weighing scale. The forage mixture was fed directly after preparation or stored in a cooling unit at 6°C to be used for future feedings for a maximum of 2 d. Forage and concentrate portions were manually mixed into a TMR for individual cows and the fresh TMR was fed twice daily at 0500 and 1530 h. Before providing fresh feed, feed refusals were collected and weighed to determine daily feed intake. For a 60-h period over d 3 to 6 of each experimental period (1700 h on d 3 until 0500 h on d 6), cows were fed using an automated feeding system that dispensed equal portions of feed every 2 h to promote metabolic steady-state conditions in preparation for the blood sampling protocol described later. Cows had free access to clean drinking water throughout the entire experiment.

### Abomasal Infusions

The abomasal infusion treatments followed a 3 × 2 factorial design, with 3 levels of corn starch and 2 levels of BHB, and contained corn starch, BHB, or a combination of both. The abomasal infusion treatments were (1) 0.0 kg of corn starch/d + 0.0 mol BHB/d, (2) 0.0 kg of corn starch/d + 8.0 mol BHB/d, (3) 1.5 kg of corn starch/d + 0.0 mol BHB/d, (4) 1.5 kg of corn starch/d + 8.0 mol BHB/d, (5) 3.0 kg of corn starch/d + 0.0 mol BHB/d, and (6) 3.0 kg of corn starch/d + 8.0 mol BHB/d (Table 2). We based the dose of BHB on the assumption that, on average, 28 kg of body fat would be mobilized over a period of 8 wk (i.e., 500 g of body fat/d; Tamminga et al., 1997; van Knegsel et al., 2007a), resulting in the production of 6.3 mol BHB/d. To increase the likelihood of inducing metabolic acidosis, the BHB dose in the present study is about 25% more than the estimated level of endogenous BHB production. With the corn starch dose, we aimed to achieve an amount of starch entering the small intestine (i.e., abomasally infused corn starch plus starch from basal TMR) that was within the range of total postru-

**Table 1.** Chemical composition (g/kg of DM, unless stated otherwise) of the TMR ingredients (grass silage, corn silage, and concentrates; analyzed) and of the complete TMR[1] (calculated)

| Item | Grass silage[2] | Corn silage[3] | Concentrate[4] | TMR |
|---|---|---|---|---|
| DM (g/kg of product) | 404 | 310 | 887 | 364 |
| OM | 899 | 953 | 903 | 920 |
| CP | 195 | 97 | 187 | 156 |
| Crude fat | 45 | 31 | 39 | 38 |
| Gross energy (MJ/kg of DM) | 19.6 | 19.1 | 18.0 | 19.0 |
| NDF | 405 | 383 | 235 | 350 |
| ADF | 214 | 230 | 120 | 194 |
| ADL | 7 | 12 | 12 | 10 |
| Starch | —[5] | 270 | 170 | 148 |
| Sugar | 120 | —[5] | 112 | 73 |
| $NE_L$[6] (MJ/kg of DM) | 6.9 | 6.8 | 7.5 | 7.0 |
| DVE[7] | 73 | 57 | 162 | 92 |
| OEB[8] | 54 | −36 | −44 | −7 |

[1]The TMR was composed of 31.5% grass silage, 43.9% corn silage, 11.3% concentrate, and 13.3% water on a product basis, which equals 350 g/kg DM grass silage, 374 g/kg DM corn silage, and 276 g/kg DM concentrate.

[2]Ensiling characteristics: acetic acid = 7 g/kg DM, lactic acid = 18 g/kg DM, ammonia-N = 7% total N, and pH = 5.8.

[3]Ensiling characteristics: acetic acid = 11 g/kg DM, lactic acid = 63 g/kg DM, ammonia-N = 6% total N, and pH = 3.8.

[4]Concentrate contained (g/kg DM): sugar beet pulp = 325, barley = 300, rumen-protected soybean meal (Mervobest, NuScience) = 200, palm kernel flakes = 60, molasses = 50, sunflower oil = 20, $CaCO_3$ = 15, MgO = 8.0, trace mineral and vitamin mix = 8.0, NaCl = 7.0, $NaHCO_3$ = 2.0, and $TiO_2$ = 5.0.

[5]Not determined.

[6]van Es, 1978.

[7]Intestinal digestible protein (van Duinkerken et al., 2011).

[8]Rumen-degraded protein balance (van Duinkerken et al., 2011).

minal starch digestion reported for lactation dairy cows fed large amounts of corn grain (maximum of 5.0 kg/d; Reynolds et al., 1997), but which would (according to van Gastelen et al., 2021) likely result in hindgut acidosis. The treatments were administered in 20-L batches that were replenished daily and infused at a rate ranging from 868 to 1,024 g/h (Table 2) to facilitate 5 d of continuous infusion (0900 h on d 1 until 0900 h on d 6 of each experimental period). To compensate the cation $Na^+$ of Na-BHB (potentially affecting the acid-base status of the cows when not compensated), 8 mol of $NH_4Cl$ was added to the Na-BHB containing treatments, where $Cl^-$ could function as the compensating anion. To compensate for the salts in the Na-BHB containing treatments (Na of Na-BHB and Cl of $NH_4Cl$; potentially affecting the water balance of the cows when not compensated), 8 mol NaCl was added to the treatments without Na-BHB. The infusion treatments were subsequently designed to be isonitrogenous, using urea to compensate the N content of $NH_4Cl$ and corn starch. Unfortunately, final infused treatments were not completely iso-N, where the amount of N infused varied between 113.0 and 113.9 g/d (Table 2). To each treatment, 48 g of Co-EDTA/d was added as a marker for intestinal permeability [method adopted from Amado et al. (2019) and Wilms et al. (2019)]. Eighty grams of carboxymethylcellulose/d (Acros Organics) was added to increase the viscosity of the treatments (i.e., to minimize settlement of particles) to facilitate homogeneous infusions.

The abomasal infusion technique was identical to the technique described by van Gastelen et al. (2021). Briefly, infusion lines were placed in the abomasum via the ruminal cannula 2 d before the first experimental period and were checked twice daily for patency (i.e., by checking the peristaltic pumps, the realized amount infused in comparison with the target amount at that moment, and the infusion line, inside and outside the cows for knits or knots) and position (i.e., by opening the rumen fistula and following the infusion line to check placement in the abomasum). The infusion device was constructed from 200 cm of braided polyvinyl chloride hose attached to the rumen cannula plug at the proximal end and a flexible disk (equipped with holes to allow digesta passage) at the distal end to secure its placement through the sulcus omasi. The flexible disk was 12 cm in diameter and made of plastisol (Bar Diamond Inc.). The infusion lines (Tygon S3 E-3603, 3.2 mm i.d., 6.4 mm o.d. for the treatments without corn starch; Tygon S3 E-3603, 4.8 mm i.d., 8.0 mm o.d. for treatments with corn starch; VWR) were connected between the treatment solutions and the multichannel peristaltic pumps with luer-to-tubing connectors. The infusion lines were subsequently inserted into the

**Table 2.** Composition of treatments that were infused into the abomasum of early-lactation dairy cows (target and realized)

| | Target (g/d, unless stated otherwise) | | | | | | | Realized (g/d, unless stated otherwise) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatment[1] (per d) | BHB | Corn starch[2] | Urea | $NH_4Cl$ | NaCl | Water (L/d) | Infusion rate (g/h) | Success rate (%) | BHB | Corn starch |
| 0.0 kg of corn starch | 0 | 0 | 244.1 | 0.0 | 467.5 | 20 | 868 | 101.4 | 0 | 0 |
| 0.0 kg of corn starch + 8.0 mol BHB | 1,017 | 0 | 4.1 | 427.9 | 0.0 | 20 | 899 | 98.8 | 1,005 | 0 |
| 1.5 kg of corn starch | 0 | 1,500 | 242.1 | 0.0 | 467.5 | 20 | 931 | 95.7 | 0 | 1,436 |
| 1.5 kg of corn starch + 8.0 mol BHB | 1,017 | 1,500 | 2.1 | 427.9 | 0.0 | 20 | 961 | 96.1 | 977 | 1,442 |
| 3.0 kg of corn starch | 0 | 3,000 | 240.0 | 0.0 | 467.5 | 20 | 993 | 96.4 | 0 | 2,892 |
| 3.0 kg of corn starch + 8.0 mol BHB | 1,017 | 3,000 | 0.0 | 427.9 | 0.0 | 20 | 1,024 | 100.0 | 1,017 | 3,000 |

[1]48 g of Co-EDTA and 80 g of carboxymethylcellulose (Acros Organics) were added to each treatment.
[2]Chemical composition (in g/kg DM, unless stated otherwise): DM = 875 g/kg, OM = 999, CP = 2.5, crude fat = 6.1, NDF = 0, ADF = 0, ADL = 0, starch = 934, sugar = 0, and gross energy = 17.5 MJ/kg DM.

braided polyvinyl chloride hose feeding into the rumen cannula. Treatments containing corn starch were maintained in suspension by continuous stirring.

### Climate Respiration Chambers

A detailed description of the CRC design and gas measurements has been reported by Heetkamp et al. (2015) and van Gastelen et al. (2015). Briefly, the relative humidity and temperature in each CRC compartment was maintained at 80% and 10°C, respectively. The CRC compartments were separated with thin walls and windows to allow audio-visual contact between cows to minimize the effects of social isolation on cow behavior, feed intake, and milk production. Cows were exposed to 16.5 h of light per d (0500 to 2130 h).

Gas concentrations and ventilation rates were corrected for pressure, temperature, and relative humidity to obtain standard temperature pressure dew point volumes of inlet and exhaust air. The inlet and exhaust air of each CRC were sampled as described by van Gastelen et al. (2015), with a second gas analyzer used for the additional 2 CRC compartments as described by Nichols et al. (2019). Production of $CO_2$ and $CH_4$ and consumption of $O_2$ were calculated from the difference between inlet and exhaust gas volumes. Once daily, calibration gases were sampled for gas analysis instead of the inlet air, and the analyzed and actual values of these calibration gases were used to correct the measured gas concentrations from the inlet air and exhaust air of the 6 CRC compartments. At the start and the end of the experiment, each CRC was checked by releasing known amounts of $CO_2$ and comparing these values with the data from the gas analysis system to determine $CO_2$ recovery. The average recovery of $CO_2$ was 100.1% (ranging between 99.5 and 100.7% for individual compartments). Gas measurements during time points when staff entered the CRC compartments (maximum 30 min for milking, feeding, and checking the abomasal infusion lines) were discarded from the data analysis. Production of $CO_2$ and $CH_4$ and consumption of $O_2$ were assumed to be linear between the last data point before opening and the first data point after closing the CRC.

### Sample Collection and Measurements

Cows were milked twice daily at 0500 and 1530 h during the adaptation and experimental periods. Milk yield was recorded at each milking. Milk samples were collected at each milking (n = 6) during the last 72 h of the infusion period into tubes containing sodium azide (5 μL) for preservation, and were stored no longer than 4 d at 4°C until analysis. An additional milk sample, representative to the milk production (i.e., 5 g sample per kg of milk produced), was collected at each milking from each cow during the last 72 h of the infusion period, pooled per cow, and stored at −20°C until energy and N analyses.

Fecal samples were collected immediately before each milking (n = 6) during the last 72 h of the infusion period by rectal grab sampling. Feces was scored for consistency using a fecal consistency scale (1–5 scale) according to Ireland-Perry and Stallings (1993) and Zaaijer et al. (2001), where 1 = runny, liquid consistency, spreads readily; 2 = thin, soft, and loose consistency, may pile slightly and spreads moderately; 3 = soft and loose, but slightly firm (not hard) consistency, piles but spreads slightly; 4 = thick and firm consistency, piles and hardly spreads; and 5 = dry: hard, dry, and stiff consistency, original form not distorted. Fecal samples were pooled into a composite sample by cow and infusion period, which was stored at −20°C until analysis for composition (i.e., for determination of ATTD of nutrients as well as energy and N balance).

Before the last 2 milking events of the infusion period, feces, urine, and ruminal fluid samples were collected. The fecal sample (~250 g) was collected by rectal grab sampling and the urine sample was collected by stimulating the dairy cows to urinate by rubbing the perineum using a vertical movement. The ruminal fluid sample (~200 mL) was obtained as described by van Zijderveld et al. (2011), and was composed of equal volumes collected from the front and middle of the ventral sac and from the cranial sac of the rumen. Immediately after collection, each sample was mixed and their pH measured using an electronic pH meter (HI9024C, Hanna Instruments). Subsequently, the urine sample was discarded and a subsample of feces (~100 g) was collected. A subsample of the ruminal fluid (600 μL) was collected and acidified with an equal volume of 0.85 mg/L orthophosphoric acid containing 19.68 m*M* isocaproic acid as an internal standard. The subsamples of feces and ruminal fluid (2 subsamples each per cow per infusion period, representing the last 2 milking events) were stored at −20°C until VFA analysis.

Samples of grass silage, corn silage, and concentrate were collected 3 times weekly during feed preparation. These samples were pooled per experimental period and stored at −20°C until analysis. During the last 72 h of the infusion period in the CRC, feed refusals were collected twice daily (0500 and 1530 h), weighed, and stored at 4°C. At the end of each experimental period, these daily orts were pooled per cow, mixed, subsampled, and stored at −20°C until analysis.

Measurements of $CH_4$ and $CO_2$ production and $O_2$ consumption were based on data recorded from d 4 (0800 h) through d 6 (0800 h) of each experimental period, whereas energy and N balance and the ATTD of nutrients were based on manure and fecal samples collected from d 3 (1000 h) through d 6 (0900 h). Cows were weighed at the start and at the end of each balance period. Each CRC compartment was cleaned at 0900 h on d 3 (taking approximately 60 min) to remove manure collected from the end of the previous period to facilitate a new total collection period. The manure (feces and urine) produced during the balance period (i.e., 1000 h on d 3 through 0900 h on d 6) was totally collected, weighed, mixed, subsampled, and stored at −20°C until analysis. To quantify the contribution of N from volatilized ammonia appearing from excreted and mixed feces and urine, samples of condensed water from the chamber heat exchanger and from a 25% sulfuric acid solution (wt/wt), through which aerial ammonia in outflowing air was trapped, were collected. This is according to Mesgaran et al. (2020). The total amount of condensed water was recorded and a representative sample was collected at the end of each balance period for each CRC compartment. For the 25% sulfuric acid solution, we recorded per each balance period and for each CRC compartment the amount of air flowing through and the weight of the sample collected. Both types of samples were stored at 4°C until analysis.

On d 5 of each experimental period, blood samples were collected from the coccygeal vessels (assumed mixture of arterial and venous blood with negligible metabolism across the tissues of the tail; Emery et al., 1965) into 10-mL sodium heparin and potassium EDTA Vacutainers (Becton Dickinson) at 0800, 1000, 1200, and 1400 h. At each sampling moment, a subsample was collected immediately from the heparin Vacutainer and analyzed by a handheld VetScan i-STAT 1 analyzer (ABAXIS Inc.) using disposable G3+ cartridges [analyzing whole blood for pH, partial pressure $CO_2$ (mmHg), partial pressure $O_2$ (mmHg), base excess (mmol/L) $HCO_3$ (mmol/L), total $CO_2$ (mmol/L), and $O_2$ saturation (%); Abbott] and CHEM 8+ cartridges [analyzing whole blood for Na (mmol/L), K (mmol/L), Cl (mmol/L), ionized Ca (mmol/L), glucose (mg/dL), urea nitrogen (mg/dL), creatinine (mg/dL), hematocrit (% PCV), hemoglobin (g/dL), and anion gap (mmol/L); Abbott]. Immediately after the subsample was collected, the heparin and potassium EDTA Vacutainers were placed in ice and subsequently centrifuged at 5,000 × *g* for 15 min at room temperature. Plasma was aliquoted by time point and stored at −20°C until further analysis (where each time point was analyzed separately).

### Analytical Procedures

Samples of grass silage, corn silage, concentrate, feed refusals, manure, and feces were thawed at room temperature, freeze-dried until a constant weight was reached, and ground to pass a 1-mm screen using a cross beater mill for the grass silage, corn silage, and feed refusals (Peppink 100AN) and an ultra-centrifugal mill for all other samples (Retsch ZM200, Retsch GmbH). Wet chemical analysis for DM, ash, N, $NH_3$-N, starch, reducing sugars (i.e., all carbohydrates with reducing properties and soluble in 40% ethanol), crude fat, NDF, ADF, ADL, and Ti was performed as described by Nichols et al. (2018). Bomb calorimetry (ISO 9831; International Organization for Standardization, 1998; adiabatic bomb calorimeter, IKA-C700, Janke and Kunkel) was used to determine gross energy (**GE**) content. Crude protein was calculated as N × 6.25.

Grass silage, corn silage, and concentrate were analyzed for DM, ash, N, crude fat, starch (corn silage and concentrate only), sugars (grass silage and concentrate only), NDF, ADF, ADL, GE, and Ti (concentrate only). Feed refusals were analyzed for DM. Manure samples were analyzed for DM, N, and GE. Fecal samples were analyzed for DM, ash, N, crude fat, starch, NDF, GE, and Ti. In addition, samples of condensed water and the sulfuric acid solution were analyzed for N. Milk samples from individual milking events were analyzed for fat, protein, lactose, urea, and BHB content by mid-infrared spectroscopy (ISO 9622; International Organization for Standardization, 2013; Vereniging Veehouderijbelangen). Pooled milk samples were analyzed for GE and N in fresh material as described above. Samples of ruminal fluid and feces were analyzed for VFA as described by van Gastelen et al. (2021).

Blood plasma was analyzed by University Veterinary Diagnostic Laboratory (Utrecht, the Netherlands) for urea, insulin, BHB, NEFA, albumin, serum amyloid A (**SAA**), and haptoglobin. Plasma NEFA, BHB, insulin, and urea were analyzed as described by van Knegsel et al. (2007b). Plasma SAA, haptoglobin, and albumin were analyzed as described by van Gastelen et al. (2021). The intraassay coefficients of variation for SAA, haptoglobin, and albumin, were 5.0, 5.3, and 0.8%, respectively. Algemeen Medisch Laboratorium (Antwerp, Belgium) analyzed blood plasma for cobalt content using inductively coupled plasma MS and a NexION 350D ICP-MS (Perkin Elmer).

### Calculations and Statistical Analysis

Reported values for nutrient content of the TMR were calculated from ration composition and analyzed values

obtained for grass silage, corn silage, and concentrate. For the grass silage and corn silage, intestinal digestible protein, rumen degradable protein balance, and $NE_L$ contents were calculated based on the chemical composition as obtained by near-infrared spectroscopy analysis (Eurofins Agro, Wageningen, the Netherlands). For the concentrate, intestinal digestible protein, rumen degradable protein balance, and $NE_L$ contents were calculated based on table values for composition of the ingredients (CVB, 2018). The ATTD of nutrients was calculated taking into account nutrient inflow from the TMR and the treatment infusions.

All variables related to feed intake, milk yield, and milk composition were averaged over the 3-d balance period. Whole-blood constituents measured with the i-STAT as well as the plasma constituents, ruminal and fecal VFA, and ruminal, fecal, and urine pH were averaged over the analyzed sampling times. The variables related to energy and N balance were expressed per kilogram of metabolic BW ($\mathbf{BW}^{0.75}$) per day. Cow was considered the experimental unit for all variables. Data were analyzed using the MIXED procedure in SAS (version 9.4, SAS Institute Inc.). The model contained main effects and interaction effects of infusion treatment factors (BHB and corn starch) as fixed effects, and cow and period as random effects. Differences were considered significant at $P \leq 0.050$ and tendencies at $0.050 < P \leq 0.100$. Multiple comparisons between treatment means were made using the Tukey-Kramer method when an effect of corn starch or a corn starch × BHB interaction was detected at $P \leq 0.050$. Treatment arrangement within the Latin square was balanced for first-order carryover effects in subsequent periods (Williams, 1949), where each treatment immediately preceded and followed every other treatment exactly once. We observed carryover effects between periods, assessed by testing for an effect of the previous treatment in the ANOVA, for blood Na (mmol/L; $P = 0.023$), anion gap (mmol/L; $P = 0.013$), and SAA (mg/L; $P = 0.003$).

## RESULTS

### Nutrient Intake and Digestibility

Nutrient intake and ATTD of nutrients were not affected by corn starch × BHB interactions, with the exception of DMI of the infusate (tendency only; $P = 0.088$) and the ATTD of starch ($P = 0.037$; Table 3). The ATTD of starch increased in the presence of BHB, but only when combined with 3.0 kg of corn starch/d.

Regardless of BHB infusion, TMR DMI was lower ($P = 0.024$) with infusion of 3.0 kg of starch/d compared with 0.0 kg of starch/d, with both not differing from 1.5 kg of starch/d. The same was observed for the intake of CP, NDF, digestible NDF, ADF, ADL, and sugar, where the intake of all these nutrients was lower ($P < 0.025$) with infusion of 3.0 kg of corn starch/d compared with 0.0 kg of corn starch/d, and with both not differing from infusion of 1.5 kg of corn starch/d. Abomasal infusion of corn starch increased the DMI of the infusate ($P < 0.001$) due to the design of the infusion treatments. Subsequently, total DMI (DMI TMR + DMI infusate) and the intake of OM and GE were higher ($P < 0.016$) with infusion of 3.0 kg of corn starch/d compared with 0.0 kg of corn starch/d, with both not differing from 1.5 kg of corn starch/d. The intake of starch increased ($P < 0.001$) with each increasing level of abomasal infusion of corn starch. The ATTD of DM, OM and GE decreased ($P < 0.001$) with each increasing level of infused corn starch. The ATTD of CP was lower ($P < 0.001$) for 1.5 kg and 3.0 kg of corn starch/d compared with 0.0 kg of corn starch/d, with no difference between 1.5 and 3.0 kg of corn starch/d.

Regardless of the presence of corn starch in the infusion, DMI of the infusate increased ($P < 0.001$) upon abomasal infusion of BHB due to the design of the infusion treatments. Infusion of BHB decreased ($P < 0.044$) the intake of digestible NDF, ATTD of CP, and ATTD of crude fat compared with no BHB infusion.

### Milk Production and Composition

Milk yield ($P = 0.075$) and milk protein concentration ($P = 0.090$) tended to be affected and feed efficiency (kg of fat- and protein-corrected milk/kg of total DMI) was affected ($P = 0.010$) by a corn starch × BHB interaction (Table 4). Abomasal infusion of 3.0 kg of corn starch/d decreased feed efficiency, but only when BHB was not present. Regardless of BHB infusion, abomasal infusion of corn starch tended to decrease ($P = 0.084$) milk fat concentration and decreased ($P < 0.001$) milk urea concentration. Milk urea concentration was lower with infusion of 3.0 kg of corn starch/d compared with 0.0 kg of corn starch/d, with both not differing from 1.5 kg of corn starch/d. Regardless of the presence of corn starch in the infusion, milk urea concentration decreased ($P = 0.007$) and milk BHB concentration increased ($P < 0.001$) with abomasal infusion of BHB compared with no BHB infusion.

### Blood and Plasma Constituents

Blood partial pressure of $O_2$ and anion gap tended ($P \leq 0.093$; Table 5) to be affected by a corn starch × BHB interaction. The results of the anion gap should be interpreted with caution because, as mentioned earlier, this variable was also significantly affected by a carryover effect between periods. Regardless of the

**Table 3.** Intake and apparent total-tract digestibility of nutrients in early-lactation dairy cows receiving abomasal infusions of corn starch, BHB, or a combination of both

| | Corn starch (kg/d) | | | BHB (mol/d) | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|
| Item | 0.0 | 1.5 | 3.0 | 0.0 | 8.0 | SEM | Starch | BHB | Starch × BHB |
| Nutrient intake (TMR + abomasal infusions, kg/d unless stated otherwise) | | | | | | | | | |
| DM TMR | 17.2$^{a}$ | 16.9$^{ab}$ | 16.0$^{b}$ | 16.9 | 16.5 | 0.70 | 0.024 | 0.146 | 0.849 |
| DM infusate | 1.19$^{a}$ | 2.40$^{b}$ | 3.76$^{c}$ | 2.07 | 2.83 | 0.024 | <0.001 | <0.001 | 0.088 |
| DM total | 18.4$^{a}$ | 19.2$^{ab}$ | 19.8$^{b}$ | 19.0 | 19.3 | 0.70 | 0.006 | 0.403 | 0.922 |
| OM | 16.4$^{a}$ | 17.2$^{ab}$ | 17.6$^{b}$ | 16.9 | 17.2 | 0.63 | 0.010 | 0.300 | 0.906 |
| Digestible OM | 12.7 | 12.8 | 12.7 | 12.6 | 12.8 | 0.47 | 0.737 | 0.220 | 0.849 |
| CP | 3.41$^{a}$ | 3.32$^{ab}$ | 3.22$^{b}$ | 3.35 | 3.28 | 0.115 | 0.017 | 0.172 | 0.919 |
| Crude fat | 0.654 | 0.649 | 0.626 | 0.652 | 0.634 | 0.0289 | 0.163 | 0.154 | 0.885 |
| Gross energy (MJ/d) | 338.3$^{a}$ | 353.1$^{ab}$ | 361.4$^{b}$ | 347.5 | 354.4 | 13.26 | 0.016 | 0.260 | 0.910 |
| NDF | 6.07$^{a}$ | 5.95$^{ab}$ | 5.67$^{b}$ | 5.98 | 5.81 | 0.280 | 0.025 | 0.155 | 0.881 |
| Digestible NDF | 4.43$^{a}$ | 4.22$^{ab}$ | 4.05$^{b}$ | 4.33 | 4.14 | 0.188 | 0.005 | 0.031 | 0.663 |
| ADF | 3.37$^{a}$ | 3.30$^{ab}$ | 3.14$^{b}$ | 3.32 | 3.22 | 0.154 | 0.024 | 0.155 | 0.873 |
| ADL | 0.174$^{a}$ | 0.171$^{ab}$ | 0.163$^{b}$ | 0.172 | 0.167 | 0.0086 | 0.025 | 0.148 | 0.789 |
| Starch | 2.51$^{a}$ | 3.64$^{b}$ | 4.75$^{c}$ | 3.66 | 3.61 | 0.108 | <0.001 | 0.378 | 0.919 |
| Sugar | 1.26$^{a}$ | 1.23$^{ab}$ | 1.17$^{b}$ | 1.24 | 1.20 | 0.055 | 0.019 | 0.121 | 0.641 |
| Apparent digestibility (% of intake) | | | | | | | | | |
| DM | 76.2$^{a}$ | 74.3$^{b}$ | 72.1$^{c}$ | 74.1 | 74.3 | 0.73 | <0.001 | 0.792 | 0.390 |
| OM | 77.2$^{a}$ | 74.7$^{b}$ | 72.0$^{c}$ | 74.5 | 74.7 | 0.77 | <0.001 | 0.692 | 0.439 |
| CP | 72.5$^{a}$ | 67.5$^{b}$ | 67.8$^{b}$ | 69.7 | 68.8 | 0.58 | <0.001 | 0.044 | 0.157 |
| Crude fat | 59.8 | 57.0 | 58.0 | 59.6 | 56.9 | 1.79 | 0.177 | 0.031 | 0.534 |
| Gross energy | 74.4$^{a}$ | 72.1$^{b}$ | 70.3$^{c}$ | 72.2 | 72.3 | 0.80 | <0.001 | 0.840 | 0.415 |
| NDF | 73.0 | 70.9 | 71.8 | 72.4 | 71.4 | 1.04 | 0.108 | 0.218 | 0.448 |
| Starch[1] | 97.9 | 97.6 | 90.6 | 95.0 | 95.7 | 0.50 | <0.001 | 0.269 | 0.037 |

$^{a–c}$Least squares means within a row with a different superscript indicate a significant difference ($P < 0.05$) between 0.0, 1.5, and 3.0 kg of corn starch/d.

[1]Starch × BHB interaction: 0.0 kg/d corn starch + 0.0 mol/d BHB = 98.1$^{x}$, 0.0 kg/d corn starch + 8.0 mol/d BHB = 97.8$^{x}$, 1.5 kg/d corn starch + 0.0 mol/d BHB = 97.9$^{x}$, 1.5 kg/d corn starch + 8.0 mol/d BHB = 97.2$^{x}$, 3.0 kg/d corn starch + 0.0 mol/d BHB = 89.0$^{y}$, 3.0 kg/d corn starch + 8.0 mol/d BHB = 92.2$^{z}$; a different superscript (x–z) indicates a significant difference ($P < 0.05$).

**Table 4.** Lactation characteristics of early-lactation dairy cows receiving abomasal infusions of corn starch, BHB, or a combination of both

| | Corn starch (kg/d) | | | BHB (mol/d) | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|
| Item | 0.0 | 1.5 | 3.0 | 0.0 | 8.0 | SEM | Starch | BHB | Starch × BHB |
| Milk yield (kg/d) | 23.1 | 23.9 | 23.5 | 23.3 | 23.7 | 1.75 | 0.373 | 0.397 | 0.075 |
| FPCM[1] (kg/d) | 24.1 | 24.5 | 23.8 | 23.9 | 24.3 | 1.31 | 0.312 | 0.346 | 0.107 |
| Feed efficiency[2] (kg of FPCM/kg of DMI total) | 1.31 | 1.27 | 1.20 | 1.26 | 1.26 | 0.047 | <0.001 | 0.896 | 0.010 |
| Fat concentration (g/100 g) | 4.42 | 4.27 | 4.16 | 4.28 | 4.29 | 0.124 | 0.084 | 0.869 | 0.578 |
| Protein concentration (g/100 g) | 3.37 | 3.37 | 3.35 | 3.38 | 3.35 | 0.152 | 0.668 | 0.174 | 0.090 |
| Lactose concentration (g/100 g) | 4.67 | 4.70 | 4.69 | 4.70 | 4.67 | 0.055 | 0.691 | 0.374 | 0.109 |
| Urea concentration (mg/dL) | 22.9[a] | 19.2[b] | 18.7[b] | 21.2 | 19.3 | 0.76 | <0.001 | 0.007 | 0.553 |
| Fat yield (g/d) | 1,005 | 1,006 | 964 | 982 | 1,001 | 48.4 | 0.149 | 0.344 | 0.178 |
| Protein yield (g/d) | 773 | 797 | 777 | 780 | 785 | 42.9 | 0.444 | 0.763 | 0.527 |
| Lactose yield (g/d) | 1,078 | 1,119 | 1,101 | 1,094 | 1,104 | 79.9 | 0.398 | 0.667 | 0.104 |
| BHB (mmol/L) | 0.082 | 0.073 | 0.073 | 0.061 | 0.091 | 0.0081 | 0.198 | <0.001 | 0.811 |

[a,b]Least squares means within a row with a different superscript indicate a significant difference ($P < 0.05$) between 0.0, 1.5, and 3.0 kg of corn starch/d.

[1]Fat- and protein-corrected milk = (0.337 + 0.116 × fat % + 0.06 × protein %) × milk yield (kg/d); CVB, 2018.

[2]Starch × BHB interaction: 0.0 kg/d corn starch + 0.0 mol/d BHB = 1.32[x]; 0.0 kg/d corn starch + 8.0 mol/d BHB = 1.29[x]; 1.5 kg/d corn starch + 0.0 mol/d BHB = 1.30[x]; 1.5 kg/d corn starch + 8.0 mol/d BHB = 1.25[x]; 3.0 kg/d corn starch + 0.0 mol/d BHB = 1.16[y]; 3.0 kg/d corn starch + 8.0 mol/d BHB = 1.24[x]; a different superscript (x,y) indicates a significant difference ($P < 0.05$).

presence of BHB, total $CO_2$ tended to decrease ($P = 0.072$) and ionized calcium tended to increase ($P = 0.074$) upon infusion of corn starch. Blood pH and $HCO_3$ were lower ($P \leq 0.048$) with infusion of 3.0 kg of corn starch/d compared with 0.0 kg of corn starch/d, with both not differing from 1.5 kg of corn starch/d. Blood base excess was lower ($P = 0.006$) for 3.0 kg of corn starch/d compared with 0.0 and 1.5 kg of corn starch/d, with no difference between the latter 2 levels of corn starch. Blood urea N was lower ($P < 0.001$) for 1.5 and 3.0 kg of corn starch/d compared with 0.0 kg of corn starch/d, with no difference between the former 2 levels of corn starch. Blood chloride concentration was higher ($P = 0.006$) with infusion of 3.0 kg of corn starch/d compared with 0.0 kg of corn starch/d, with both not differing from 1.5 kg of corn starch/d.

Regardless of the presence of corn starch in the infusion, BHB infusion decreased ($P \leq 0.031$) blood base excess; blood concentration of $HCO_3$, total $CO_2$, and glucose; and plasma concentration of insulin, compared with no BHB infusion. Infusion of BHB increased ($P \leq 0.045$) blood anion gap, and plasma BHB and cobalt concentration compared with no BHB infusion. Also here, the results of the anion gap should be interpreted with caution because this variable was also significantly affected by a carryover effect between periods.

### Energy and Nitrogen Balance

None of the variables associated with energy and N balance were affected by a corn starch × BHB interaction (Table 6). Regardless of the presence of BHB, metabolic BW tended to decrease in response to corn starch infusion. The respiratory quotient was higher ($P < 0.001$) with infusion of 1.5 and 3.0 kg of corn starch/d compared with 0.0 kg of corn starch/d, with no difference between the former 2 levels of corn starch. Intake of GE (**GEI**) was higher ($P = 0.013$) with infusion of 3.0 kg of corn starch/d compared with 0.0 kg of corn starch/d, with both not differing from 1.5 kg of corn starch/d. Energy excreted in manure increased ($P < 0.001$) with every increasing level of infused corn starch. The ratio between metabolizable energy intake (**MEI**) and GEI was lower ($P < 0.001$) for 3.0 kg of corn starch/d compared with 0.0 and 1.5 kg of corn starch/d, with no difference between the latter 2 levels of corn starch.

Nitrogen intake was lower with infusion of 3.0 kg of corn starch/d compared with 0.0 kg of corn starch/d, with both not differing from 1.5 kg of corn starch/d. Nitrogen excreted via urine and N trapped in condensed water and acid were higher ($P \leq 0.007$) for 0.0 kg of corn starch/d compared with 1.5 and 3.0 kg of corn starch/d, with no difference between the latter 2 levels

**Table 5.** Blood and plasma constituents of early-lactation dairy cows receiving abomasal infusions of corn starch, BHB, or a combination of both

| | Corn starch (kg/d) | | | BHB (mol/d) | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|
| Item | 0.0 | 1.5 | 3.0 | 0.0 | 8.0 | SEM | Starch | BHB | Starch × BHB |
| Blood constituent | | | | | | | | | |
| pH | 7.43[a] | 7.42[ab] | 7.40[b] | 7.42 | 7.41 | 0.011 | 0.028 | 0.283 | 0.155 |
| $pCO_2$[1] (mm Hg) | 42.1 | 42.2 | 43.3 | 43.0 | 42.1 | 1.06 | 0.387 | 0.251 | 0.536 |
| $pO_2$[2] (mm Hg) | 75 | 85 | 68 | 76 | 76 | 13.0 | 0.125 | 0.941 | 0.075 |
| Base excess[3] (mmol/L) | 3.4[a] | 3.0[a] | 1.7[b] | 3.3 | 2.0 | 0.58 | 0.006 | 0.002 | 0.302 |
| $HCO_3$ (mmol/L) | 27.9[a] | 27.7[ab] | 26.8[b] | 28.1 | 26.9 | 0.56 | 0.048 | 0.002 | 0.516 |
| Total $CO_2$ (mmol/L) | 29 | 29 | 28 | 29 | 28 | 0.6 | 0.072 | 0.002 | 0.498 |
| $sO_2$[4] (%) | 85 | 87 | 82 | 85 | 84 | 3.2 | 0.287 | 0.627 | 0.405 |
| Sodium (mmol/L) | 139 | 139 | 139 | 139 | 139 | 0.3 | 0.246 | 0.999 | 0.288 |
| Potassium (mmol/L) | 4.7 | 4.7 | 4.9 | 4.7 | 4.8 | 0.09 | 0.134 | 0.426 | 0.562 |
| Chloride (mmol/L) | 101[a] | 101[ab] | 102[b] | 101 | 102 | 0.6 | 0.006 | 0.173 | 0.516 |
| Ionized calcium (mmol/L) | 1.26 | 1.25 | 1.28 | 1.26 | 1.26 | 0.012 | 0.074 | 0.482 | 0.435 |
| Glucose (mg/dL) | 66 | 67 | 67 | 68 | 65 | 1.9 | 0.867 | 0.006 | 0.556 |
| Hematocrit[5] (% PCV) | 26 | 25 | 25 | 25 | 25 | 0.9 | 0.298 | 0.494 | 0.619 |
| Hemoglobin (g/dL) | 8.54 | 8.49 | 8.66 | 8.65 | 8.47 | 0.332 | 0.644 | 0.256 | 0.671 |
| Urea N (mg/dL) | 13.8[a] | 11.0[b] | 11.5[b] | 12.4 | 11.8 | 1.10 | <0.001 | 0.169 | 0.774 |
| Creatinine (mg/dL) | 0.57 | 0.55 | 0.55 | 0.56 | 0.55 | 0.038 | 0.331 | 0.484 | 0.419 |
| Anion gap (mmol/L) | 18.1 | 17.6 | 18.0 | 17.6 | 18.3 | 0.41 | 0.216 | 0.015 | 0.093 |
| Plasma constituent | | | | | | | | | |
| Urea (mmol/L) | 4.3 | 4.0 | 4.2 | 4.2 | 4.1 | 0.29 | 0.269 | 0.488 | 0.961 |
| Insulin (mIU/L) | 12.6 | 12.8 | 13.0 | 13.7 | 11.9 | 2.80 | 0.940 | 0.031 | 0.154 |
| BHB (mmol/L) | 0.57 | 0.59 | 0.58 | 0.50 | 0.66 | 0.067 | 0.932 | 0.013 | 0.387 |
| NEFA[6] (mmol/L) | 0.17 | 0.17 | 0.17 | 0.18 | 0.16 | 0.015 | 0.951 | 0.208 | 0.708 |
| Albumin (g/L) | 33 | 32 | 33 | 33 | 33 | 0.8 | 0.357 | 0.591 | 0.452 |
| Serum amyloid A (mg/L) | 93 | 124 | 79 | 114 | 83 | 25.4 | 0.340 | 0.221 | 0.169 |
| Haptoglobin (g/L) | 0.69 | 0.49 | 0.70 | 0.63 | 0.62 | 0.146 | 0.475 | 0.943 | 0.917 |
| Cobalt (ug/L) | 225 | 246 | 265 | 226 | 265 | 29.8 | 0.215 | 0.045 | 0.124 |

[a,b]Least squares means within a row with a different superscript indicate a significant difference ($P < 0.05$) between 0.0, 1.5, and 3.0 kg of corn starch/d.

[1]$pCO_2$ = partial pressure $CO_2$.

[2]$pO_2$ = partial pressure $O_2$.

[3]Base excess is defined as the mEq of acid or base that must be added to 1 L of blood to restore the pH to 7.40 at 37°C and at a $pCO_2$ of 40 mmHg (Corey, 2003).

[4]$sO_2$ = $O_2$ saturation.

[5]PCV = ratio between the volume of red blood cells and the total volume of blood.

[6]NEFA = nonesterified fatty acids.

**Table 6.** The energy and nitrogen balance of early-lactation dairy cows receiving abomasal infusions of corn starch, BHB, or a combination of both

| | Corn starch (kg/d) | | | BHB (mol/d) | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|
| Item | 0.0 | 1.5 | 3.0 | 0.0 | 8.0 | SEM | Starch | BHB | Starch × BHB |
| Respiratory quotient | 1.120[a] | 1.147[b] | 1.147[b] | 1.145 | 1.131 | 0.0100 | <0.001 | 0.001 | 0.786 |
| Metabolic BW[1] ($kg^{0.75}$) | 124 | 124 | 123 | 124 | 124 | 2.3 | 0.096 | 0.306 | 0.847 |
| Energy balance (kJ/kg of $BW^{0.75}$ per day, unless stated otherwise) | | | | | | | | | |
| GEI[2] | 2,729[a] | 2,842[ab] | 2,930[b] | 2,809 | 2,859 | 110.3 | 0.013 | 0.334 | 0.923 |
| $CH_4$ production | 177 | 173 | 165 | 175 | 168 | 7.6 | 0.124 | 0.146 | 0.807 |
| Energy in manure | 854[a] | 944[b] | 1,044[c] | 950 | 945 | 46.6 | <0.001 | 0.858 | 0.858 |
| DEI[3] | 2,031 | 2,052 | 2,058 | 2,029 | 2,065 | 84.4 | 0.784 | 0.292 | 0.846 |
| MEI[4] | 1,699 | 1,726 | 1,722 | 1,685 | 1,746 | 65.0 | 0.625 | 0.022 | 0.885 |
| MEI to GEI ratio (%) | 62.3[a] | 60.8[a] | 58.8[b] | 60.1 | 61.1 | 0.64 | <0.001 | 0.106 | 0.962 |
| Heat production[5] | 957 | 961 | 958 | 952 | 966 | 28.3 | 0.987 | 0.452 | 0.999 |
| Energy in milk | 602 | 611 | 599 | 601 | 607 | 29.1 | 0.644 | 0.545 | 0.195 |
| ER total[6] | 139 | 154 | 165 | 133 | 172 | 36.5 | 0.694 | 0.120 | 0.484 |
| ER protein[7] | 73 | 60 | 61 | 70 | 59 | 9.0 | 0.523 | 0.339 | 0.383 |
| ER fat[8] | 66 | 94 | 104 | 63 | 113 | 31.9 | 0.414 | 0.048 | 0.721 |
| Nitrogen balance (mg/kg of $BW^{0.75}$ per day) | | | | | | | | | |
| N intake[9] | 4,400[a] | 4,279[ab] | 4,173[b] | 4,335 | 4,234 | 149.0 | 0.035 | 0.138 | 0.929 |
| N manure | 2,849 | 2,811 | 2,716 | 2,807 | 2,777 | 85.2 | 0.165 | 0.604 | 0.496 |
| N feces[10] | 1,211[a] | 1,388[b] | 1,344[b] | 1,310 | 1,319 | 45.7 | <0.001 | 0.767 | 0.499 |
| N urine[11] | 1,638[a] | 1,423[b] | 1,373[b] | 1,497 | 1,458 | 68.2 | 0.007 | 0.556 | 0.331 |
| N milk | 973 | 998 | 985 | 984 | 987 | 53.0 | 0.642 | 0.874 | 0.629 |
| N condensed water + N acid | 82[a] | 65[b] | 60[b] | 72 | 66 | 10.3 | <0.001 | 0.142 | 0.399 |
| N balance | 496 | 405 | 412 | 472 | 403 | 61.4 | 0.523 | 0.339 | 0.383 |
| N efficiency[12] | 22.1[a] | 23.3[b] | 23.6[b] | 22.7 | 23.3 | 0.90 | 0.004 | 0.105 | 0.185 |

[a–c]Least squares means within a row with a different superscript indicate a significant difference ($P < 0.05$) between 0.0, 1.5, and 3.0 kg of corn starch/d.

[1]The mean BW per cow per balance period was used to calculate metabolic BW ($BW^{0.75}$).

[2]GEI = gross energy intake (TMR plus infusate).

[3]DEI (digestible energy intake) = GEI × apparent total-tract digestibility of GE (% of intake).

[4]MEI (metabolizable energy intake) = GEI − methane production − energy in manure (feces + urine).

[5]Heat production (kJ/d) = 16.175 × $VO_2$ (L/d) + 5.021 × $VCO_2$ (L/d), where $VO_2$ = volumes of $O_2$ consumed, and $VCO_2$ = volumes of $CO_2$ produced (Gerrits et al., 2015).

[6]Energy retention total = MEI − heat production − energy in milk.

[7]Energy retention protein = protein gain (N × 6.25) × 23.6 kJ/g (energetic value of protein).

[8]Energy retention fat = energy retention total − energy retention protein.

[9]N intake from TMR plus infusate.

[10]N feces = N intake × [100 − apparent total-tract digestibility of N (% of intake)]/100.

[11]N urine = N manure − N feces.

[12]N efficiency = N milk/N intake (%).

of corn starch. Infusion of corn starch increased ($P \leq 0.004$) N excreted via feces and N efficiency, where both were lower with 0.0 kg of corn starch/d compared with 1.5 and 3.0 kg of corn starch/d, with no difference between the latter 2 levels of corn starch.

Regardless of the presence of corn starch, infusion of BHB decreased ($P = 0.001$) the respiratory quotient, but increased ($P \leq 0.048$) MEI and energy retained as fat compared with no BHB infusion. Abomasal infusion of BHB did not affect any of the N balance variables.

### Ruminal Fluid, Feces, and Urine

Ruminal butyrate proportions, fecal total VFA concentration, and fecal valerate proportions were affected ($P \leq 0.031$) by a corn starch × BHB interaction (Table 7). Despite the significant corn starch × BHB interaction for ruminal butyrate, no significant differences were found between treatment means using the Tukey-Kramer method. Infusion of BHB decreased the fecal total VFA concentration, but only when combined with 3.0 kg of corn starch/d. Fecal valerate proportions decreased with every increasing level of infused corn starch, but only in the absence of BHB. With BHB infusion, the fecal valerate proportion was higher for 0.0 kg of corn starch/d compared with 1.5 and 3.0 kg of corn starch/d, with no difference between the latter 2 levels of corn starch.

Regardless of the presence of BHB in the infusion, starch infusion did not affect any of the ruminal fermentation variables. Fecal consistency score was higher ($P = 0.050$) for 3.0 kg of corn starch/d compared with 0.0 kg of corn starch/d, with both levels of corn starch not differing from 1.5 kg of corn starch/d. Fecal pH and fecal isovalerate proportion decreased ($P < 0.001$) with every increasing level of corn starch infused. Fecal propionate proportion and urine pH were lower ($P < 0.001$) with 3.0 kg of corn starch/d compared with 0.0 and 1.5 kg of corn starch/d, with no difference between the latter 2 levels of corn starch. Infusion of corn starch also decreased ($P < 0.001$) fecal isobutyrate proportions, which were lower with 1.5 and 3.0 kg of corn starch/d compared with 0.0 kg of corn starch/d, with no difference between the former 2 levels of corn starch. Fecal butyrate proportions increased ($P < 0.001$) with every increasing level of infused corn starch. The acetate to propionate ratio in feces increased ($P < 0.001$) with infusion of 3.0 kg of corn starch/d compared with 0.0 and 1.5 kg of corn starch/d, with no difference between the latter 2 levels of corn starch.

Regardless of the presence of corn starch, fecal acetate proportion ($P = 0.069$) and urine pH ($P = 0.057$) tended to decrease with abomasal infusion of BHB. Fecal butyrate proportions were higher ($P = 0.009$) when BHB was present in the infusion compared with no BHB.

## DISCUSSION

The aim of this study was to induce hindgut and metabolic acidosis via abomasal infusion of corn starch and BHB, respectively, and to determine the effects of these physiological states in early-lactation cows. Ruminal fermentation characteristics were largely unaffected by either starch or BHB infusion, suggesting that there was no appreciable back-flow of infusates into the rumen. Therefore, our aim to induce acidosis only posterior to the rumen was achieved.

### Establishment of Hindgut Acidosis

Currently, there is no clear fecal pH threshold defined for hindgut acidosis, but fecal pH was previously reported to be 6.30 (Li et al., 2012) to 6.49 (Danscher et al., 2015) for control-fed animals. Considering these values as a threshold in combination with the observed fecal pH, hindgut acidosis was achieved upon infusion of 1.5 and 3.0 kg/d starch, and became more severe with a higher corn starch level. Van Gastelen et al. (2021) reported a fecal pH of 6.86 without abomasal infusion of ground corn and a fecal pH of 6.00 when 3.0 kg of ground corn/d (equals ~1.5 kg of starch/d, when corrected for DM and starch content) was infused into the abomasum. This is in agreement with the results of the present study, where infusion of 1.5 kg of corn starch/d resulted in an average fecal pH of 6.00 and infusion of 3 kg of corn starch/d decreased fecal pH even further.

The decrease in fecal pH with 1.5 and 3.0 kg of corn starch/d suggests that part of the infused corn starch escaped intestinal digestion and was fermented in the hindgut (Reynolds et al., 2001). This is supported by the decrease in ATTD of CP, likely as a result of increased microbial protein synthesis, and by the increase in respiratory quotient, likely as a result of anaerobic fermentation of dietary carbohydrates (Gerrits et al., 2015). Contrary to van Gastelen et al. (2021), we did not observe an increase in total VFA concentration in feces upon abomasal infusion of corn starch. This lack of effect on fecal total VFA concentrations combined with decreased fecal pH suggests that the buffering capacity of the large intestine may have changed with abomasal infusion of corn starch.

**Table 7.** pH and VFA of ruminal fluid, feces, and urine (pH only) of early-lactation dairy cows receiving abomasal infusions of corn starch, BHB, or a combination of both

| | Corn starch (kg/d) | | | BHB (mol/d) | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|
| Item | 0.0 | 1.5 | 3.0 | 0.0 | 8.0 | SEM | Starch | BHB | Starch × BHB |
| Rumen | | | | | | | | | |
| pH | 6.30 | 6.20 | 6.31 | 3.29 | 6.25 | 0.078 | 0.225 | 0.448 | 0.923 |
| Total VFA (m*M*) | 106 | 115 | 114 | 110 | 113 | 4.5 | 0.069 | 0.366 | 0.188 |
| VFA (% of total VFA) | | | | | | | | | |
| Acetate | 65.5 | 65.0 | 65.7 | 65.5 | 65.4 | 0.84 | 0.300 | 0.872 | 0.368 |
| Propionate | 18.9 | 19.0 | 19.0 | 19.1 | 18.8 | 0.83 | 0.943 | 0.498 | 0.147 |
| Butyrate[1] | 11.3 | 11.3 | 10.9 | 10.9 | 11.3 | 0.41 | 0.431 | 0.194 | 0.031 |
| Isobutyrate | 0.83 | 0.83 | 0.85 | 0.83 | 0.84 | 0.028 | 0.677 | 0.917 | 0.228 |
| Valerate | 1.58 | 1.65 | 1.58 | 1.60 | 1.60 | 0.062 | 0.311 | 0.989 | 0.505 |
| Isovalerate | 1.91 | 2.18 | 1.96 | 2.04 | 1.99 | 0.238 | 0.348 | 0.754 | 0.431 |
| Acetate to propionate ratio | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 0.17 | 0.919 | 0.427 | 0.265 |
| Feces | | | | | | | | | |
| Consistency | $2.5^{a}$ | $2.8^{ab}$ | $3.0^{b}$ | 2.9 | 2.6 | 0.19 | 0.050 | 0.131 | 0.186 |
| pH | $6.49^{a}$ | $6.00^{b}$ | $5.15^{c}$ | 5.95 | 5.81 | 0.094 | <0.001 | 0.132 | 0.123 |
| Total VFA[2] (m*M*) | 58 | 61 | 55 | 58 | 57 | 3.2 | 0.190 | 0.566 | 0.015 |
| VFA (% of total VFA) | | | | | | | | | |
| Acetate | 75.3 | 73.5 | 72.0 | 74.9 | 72.3 | 1.06 | 0.147 | 0.069 | 0.894 |
| Propionate | $13.4^{a}$ | $11.9^{a}$ | $4.9^{b}$ | 10.7 | 9.5 | 1.25 | <0.001 | 0.331 | 0.534 |
| Butyrate | $8.4^{a}$ | $13.4^{b}$ | $22.5^{c}$ | 12.8 | 16.7 | 1.22 | <0.001 | 0.009 | 0.305 |
| Isobutyrate | $1.12^{a}$ | $0.48^{b}$ | $0.24^{b}$ | 0.71 | 0.52 | 0.101 | <0.001 | 0.131 | 0.838 |
| Valerate[3] | 1.01 | 0.35 | 0.21 | 0.51 | 0.54 | 0.077 | <0.001 | 0.801 | 0.022 |
| Isovalerate | $0.67^{a}$ | $0.34^{b}$ | $0.05^{c}$ | 0.38 | 0.33 | 0.053 | <0.001 | 0.448 | 0.813 |
| Acetate to propionate ratio | $5.7^{a}$ | $8.1^{a}$ | $14.2^{b}$ | 9.2 | 9.5 | 1.21 | <0.001 | 0.817 | 0.330 |
| Urine | | | | | | | | | |
| pH | $7.64^{a}$ | $7.65^{a}$ | $7.38^{b}$ | 7.61 | 7.50 | 0.066 | <0.001 | 0.057 | 0.431 |

[a–c]Least squares means within a row with a different superscript indicate a significant difference ($P < 0.05$) between 0.0, 1.5, and 3.0 kg of corn starch/d.

[1]Starch × BHB interaction: 0.0 kg/d corn starch + 0.0 mol/d BHB = 11.6, 0.0 kg/d corn starch + 8.0 mol/d BHB = 10.9, 1.5 kg/d corn starch + 0.0 mol/d BHB = 10.8, 1.5 kg/d corn starch + 8.0 mol/d BHB = 11.7, 3.0 kg/d corn starch + 0.0 mol/d BHB = 10.4, 3.0 kg/d corn starch + 8.0 mol/d BHB = 11.4; no significant differences ($P > 0.05$).

[2]Starch × BHB interaction: 0.0 kg/d corn starch + 0.0 mol/d BHB = $54^{xy}$, 0.0 kg/d corn starch + 8.0 mol/d BHB = $61^{x}$, 1.5 kg/d corn starch + 0.0 mol/d BHB = $60^{xy}$, 1.5 kg/d corn starch + 8.0 mol/d BHB = $62^{x}$, 3.0 kg/d corn starch + 0.0 mol/d BHB = $61^{x}$, 3.0 kg/d corn starch + 8.0 mol/d BHB = $48^{y}$; a different superscript (x,y) indicates a significant difference ($P < 0.05$).

[3]Starch × BHB interaction: 0.0 kg/d corn starch + 0.0 mol/d BHB = $1.05^{x}$, 0.0 kg/d corn starch + 8.0 mol/d BHB = $0.97^{x}$, 1.5 kg/d corn starch + 0.0 mol/d BHB = $0.48^{y}$, 1.5 kg/d corn starch + 8.0 mol/d BHB = $0.22^{yz}$, 3.0 kg/d corn starch + 0.0 mol/d BHB = $0.01^{z}$, 3.0 kg/d corn starch + 8.0 mol/d BHB = $0.42^{yz}$; a different superscript (x–z) indicates a significant difference ($P < 0.05$).

Infusion of corn starch in the abomasum appeared to disturb the acid-base status of the dairy cows, likely related to increased VFA absorption from the hindgut during corn starch infusion. In the rumen, absorption of VFA is suspected to be primarily regulated by the anion exchange pathway, where VFA are absorbed in exchange for the secretion of $HCO_3$ (Aschenbach et al., 2009). An acid load in the rumen depleted $HCO_3$ from the blood and decreased blood pH in beef steers (Brown et al., 2000). This mechanism is likely valid for the hindgut as well, especially when excessive fermentation takes place.

The increase in the molar proportion of fecal butyrate upon abomasal infusion of corn starch is consistent with what has been observed in monogastric animals during hindgut fermentation of slowly fermentable starch (Lv et al., 2006), as well as in dairy cattle fed a high concentrate diet (Mao et al., 2012). It has been suggested that elevated colonic butyrate levels may support a healthy gut environment because of the use of butyrate by the intestinal epithelium as an energy source (Brouns et al., 2002). Increased fecal concentration of butyrate, assuming absorption of butyrate has not changed largely, is an indication of increased butyrate production in the hindgut. Thus, more butyrate may have been available for hindgut epithelia, and could have provided a supportive effect on epithelial function in the hindgut.

Based on the data from the present study and that of van Gastelen et al. (2021), we determined the relation between fecal pH and the amount of corn starch infused into the abomasum (i.e., on individual cow level, not treatment means). The Pearson correlation between fecal pH and the amount of corn starch abomasally infused was negative ($P < 0.001$, r = −0.84), where fecal pH = 6.74 (±0.063) − 0.59 (±0.047) × kg of corn starch abomasally infused. Assuming that a fecal pH of 6.00 (i.e., lower threshold reported by Plaizier et al., 2018) indicates hindgut acidosis, hindgut acidosis would be induced as soon as 1.25 kg of extra corn starch/d enters the abomasum, irrespective of the source of corn starch. Based on the variation in ruminal starch digestion in ruminants (Mills et al., 1999; Allen, 2000; Moharrery et al., 2014), we assume that 60% (i.e., common for corn) to 80% of starch intake is fermented in the rumen. Considering this alongside the above-described equation, daily total starch intake would have to reach at least 3.1 to 6.3 kg/d before hindgut acidosis may possibly be induced. The average DMI of the present study and that of van Gastelen et al. (2021) was 16.7 kg/d. Hence, the intake of 3.1 and 6.3 kg of starch/d would be achieved at a dietary starch content of 186 to 371 g/kg of DM, respectively. These levels, particularly the lower dietary starch content, is easily achieved in practice under many farming conditions.

### *Effects of Abomasal Corn Starch Infusion and Associated Hindgut Acidosis*

The model used to induce hindgut acidosis in the present study differs from some studies where hindgut acidosis was induced. Li et al. (2012) and Plaizier et al. (2017) induced hindgut acidosis via a grain-based SARA challenge. In this model, feeding large amounts of grain is combined with poor rumen mat function associated with SARA conditions. This generally leads to larger amounts of fermentable substrates that bypass rumen fermentation. If these substrates are not digested in the small intestine, these will be fermented in the hindgut (Plaizier et al., 2018). In contrast, the abomasal infusion model used in the present study induced hindgut acidosis but did not affect ruminal conditions. Hence, differences in the models applied to induce hindgut acidosis may lead to differences in results (i.e., the effects of hindgut acidosis) across studies.

***Nutrient Intake and Digestibility.*** We observed a depressed voluntary TMR DMI with abomasal infusion of 3.0 kg of corn starch/d, but not with the abomasal infusion of 1.5 kg of corn starch/d. This is in agreement with van Gastelen et al. (2021), who did not observe a decreased voluntary DMI when 3.0 kg of ground corn/d (equal to ~1.5 kg of starch/d) was infused in the abomasum. On the contrary though, both Reynolds et al. (1998) and Knowlton et al. (1998) observed a decreased voluntary DMI when 1.2 kg of wheat starch/d and 1.5 kg of hydrolyzed starch/d was infused, respectively.

In the present study, ATTD of nutrients was determined by including $TiO_2$ in the concentrate as an external marker and collecting fecal samples twice daily during the infusion period. Morris et al. (2018) stated that sampling feces twice daily (as we have done) might result in different fecal marker and nutrient concentrations compared with sampling feces 12 times daily. However, cows were fed only once daily in the study of Morris et al. (2018), whereas the cows in the present study were fed according to a steady-state pattern during the period of sampling. Steady-state feeding will reduce diurnal variation in fecal marker and nutrient concentrations (Owens and Hanson, 1992). Furthermore, Titgemeyer et al. (2001) showed that $TiO_2$ recoveries that did not differ from 100% when cattle were adapted to marker administration for 17 to 21 d before measurement of nutrient ATTD. In the present study, cows were adapted to marker administration for at least 21 d. Hence, we consider the duration the duration of marker administration as well as the frequency of fecal sampling (in combination with steady-state feeding) sufficient to draw conclusions from the results concerning ATTD of nutrients.

The decreased ATTD of most nutrients upon abomasal infusion of corn starch is generally in agreement with van Gastelen et al. (2021) but contrary to Knowlton et al. (1998) and Reynolds et al. (2001). Van Gastelen et al. (2021) proposed that overall fermentation and absorption are lower for postruminally infused nutrients than for nutrients entering the rumen. By avoiding ruminal fermentation, it is likely that starch, and subsequently, DM, OM, and GE (i.e., because starch contributes to these fractions), were digested to a lesser extent. In contrast, nutrients that originate entirely from the TMR fed (i.e., fat and NDF) undergo rumen fermentation, explaining the unaffected ATTD of these nutrients upon abomasal infusion of corn starch. Decreased ATTD of CP was likely the result of increased microbial protein synthesis upon fermentation of the infused corn starch, and was also observed by Gressley and Armentano (2007) and Gressley et al. (2011) in response to increased starch supply. If this microbial protein remains undigested in the hindgut, it is excreted as microbial N and thereby decreases ATTD of N (Owens et al., 1986). As N contributes to the DM, OM, and GE fraction, decreased ATTD of N likely also results in decreased ATTD of DM, OM, and GE.

***Energy Partitioning.*** The decreased ATTD of GE, as discussed above, in combination with increased GEI, resulted in increased energy excreted via manure upon abomasal infusion of corn starch. When corn starch is digested or fermented in the hindgut, increased MEI is expected from greater glucose and VFA absorption (Reynolds, 2006). In the present study, however, both MEI and blood glucose concentration were unaffected by abomasal infusion of corn starch, contrary to van Gastelen et al. (2021) but in line with Knowlton et al. (1998). The lack of effect on blood glucose concentration, despite the large quantities of starch that were infused, can be explained by greater utilization of arterial glucose by tissues of the portal-drained viscera relative to absorbed glucose (Reynolds et al., 1998), oxidation of luminal glucose by enterocytes (Knowlton et al., 1998), decreased hepatic gluconeogenesis in response to the increased glucose absorption (Clark et al., 1977), or a combination of these mechanisms.

Milk yield is largely regulated through the osmotic properties of lactose, where synthesis of lactose requires the transport of glucose from blood into the cytosol of mammary epithelial cells (Cant et al., 2002). The unaffected milk yield, milk lactose concentration, and lactose yield in response to abomasal corn starch infusion, are therefore in line with the increased energy excretion in manure and the unaffected blood glucose concentrations. Milk fat concentration tended to decrease when corn starch was infused into the abomasum, which is contrary to Reynolds et al. (2001), Rius et al. (2010), and van Gastelen et al. (2021), but similar to Knowlton et al. (1998). When elevated in circulation, glucose promotes insulin secretion (Bauman and Griinari, 2001). Insulin stimulates the uptake of lipogenic precursors into adipose tissue and decreases lipolysis, resulting in a reduction of circulating fatty acids for milk fat synthesis (Bauman and Griinari, 2001). Although energy retained as fat, blood glucose concentration, and concentrations of insulin, NEFA, and BHB in plasma were not affected by abomasal infusion of corn starch in the present study, the tendency for a decreased milk fat concentration suggests a reduced availability of long-chain fatty acids for milk lipid synthesis during infusion of corn starch.

***Nitrogen Partitioning.*** The transfer efficiency of feed N into milk N may increase when glucogenic energy supply to the animal is increased. This is because of reduced catabolism of AA for gluconeogenesis, leaving more available for milk protein synthesis. Contrary to the findings of Rius et al. (2010) and van Gastelen et al. (2021), milk protein content or yield did not increase in response to corn starch infusion in the present study. These results are, however, in line with the lack of effect of starch infusion on blood glucose or plasma insulin concentrations and in agreement with Knowlton et al. (1998) and Reynolds et al. (2001). The increased N efficiency observed with corn starch infusion was due to lower N intake as a result of a lower DMI of the TMR combined with unaffected milk N secretion.

We observed a clear shift in N excretion from urinary N to fecal N upon abomasal infusion of corn starch, similar to Reynolds et al. (2001) and van Gastelen et al. (2021). This is likely related to increased microbial protein synthesis in the hindgut arising from fermentation of the infused corn starch in the large intestine. The N source for this microbial protein may have partly been derived from blood next to N sources in the digesta. Thus, the decreased urinary N is in line with the decreased blood urea N concentration and decreased milk urea concentration upon abomasal infusion of corn starch.

***Immune Response.*** Similar to van Gastelen et al. (2021), the lack of effect of abomasally infused corn starch on the plasma concentration of acute phase proteins suggests that hindgut fermentation of corn starch did not result in a systemic inflammatory response detectable by these plasma markers. An alteration in the condition of intercellular tight junctions (e.g., structural damage in response to acidic conditions), can result in increased permeability of the intestine to large molecules. Damaged tight junctions become leaky, allowing pathogens and toxins to enter the bloodstream (Kameda et al., 1968). Intestinal permeability measured by the appearance of Co (of the indigestible marker Co-

EDTA) in blood appeared to be unaffected, suggesting that abomasal infusion of up to 3.0 kg of corn starch/d did not result in a leaky gut.

### Establishment of Metabolic Acidosis

Blood pH was not affected by abomasal infusion of BHB and was within the normal physiological range for BHB and non-BHB treatments (i.e., between 7.38 and 7.43 pH units; Indrova et al., 2017). This suggests that daily infusion of 8 mol BHB did not result in metabolic acidosis. However, in clinically healthy cows, acid-base disturbances are compensated by various regulatory mechanisms (Gärtner et al., 2019). Respiratory compensation is commonly characterized during suspected acid-base disturbances by a reduced partial pressure of $CO_2$ (Gant et al., 1998). Respiratory compensation, however, is of minor importance in dairy cows because of their relatively small lung capacity (Enemark et al., 2002), so it follows that $pCO_2$ was maintained within normal range (i.e., between 35 and 45 mmHg; Constable, 1999) during abomasal infusion of BHB in the present study. When a respiratory response is not present, total $CO_2$, $HCO_3$, and base excess can be used as indicators for an acid-base disturbance (Constable, 1999). Although these 3 variables decreased upon abomasal infusion of BHB, they remained within their normal physiological ranges (e.g., between −0.5 and 4.5 mmol/L for base excess; Indrova et al., 2017). These results, combined with a tendency for a decreased urinary pH, suggest that a compensated metabolic acidosis was induced via abomasal BHB infusion. This is characterized by an acid-base disturbance but un unaffected blood pH (Vagnoni and Oetzel, 1998; Zimpel et al., 2018).

Concentration greater than 1.2 mmol BHB/L of blood (Enjalbert et al., 2001) and 0.2 mmol BHB/L of milk (Geishauser et al., 2000) have been proposed as critical threshold values indicating subclinical ketosis. The observed concentrations of BHB in milk and plasma were well below these thresholds, irrespective of the infusion treatment. This suggests that 8 mol BHB/d infused into the abomasum was not sufficient to induce a state resembling subclinical ketosis or ketoacidosis (i.e., accumulation of ketone bodies causing metabolic acidosis; Ewaschuk et al., 2002). We based our BHB dose on the assumption that, on average, 28 kg of body fat would be mobilized over a period of 8 wk (i.e., 500 g of body fat/d; Tamminga et al., 1997; van Knegsel et al. 2007a). Mobilization of 500 g of body fat/d equals some 1.6 mol C18 fatty acids/d, considering the molar weight of triglycerides (i.e., 946 g if consisting of glycerol and 3 fatty acids with 18C). We assumed that 100% of these C18 fatty acids would be converted into BHB as a C4 fatty acid (i.e., 1 mol C18 fatty acids delivering 4 mol BHB), and that the capacity of the liver to oxidize NEFA is exceeded and all NEFA are converted into ketone bodies. Hence, 6.3 mol BHB/d would be produced by the liver on average for the first 8 wk of lactation. In the current study, we realized an infused supply of 7.7 to 8.0 mol BHB/d, which is about 25% more than this estimated level of endogenous BHB production. This dose was apparently not sufficient to induce a subclinical ketosis or ketoacidosis. Herrick et al. (2018) stated that ketosis-indicating threshold concentrations of BHB in milk or plasma are based on elevated levels of endogenously produced ketones arising from liver dysfunction and excessive body fat mobilization. There was no evidence in the current study that these conditions were occurring upon BHB infusion (i.e., increased energy retention in body fat, no effect on plasma NEFA concentrations or TMR DMI). Therefore, it is logical that exogenously supplied BHB produced a metabolic response characterized as a compensated metabolic acidosis rather than a classical ketoacidosis.

### Effects of Abomasal BHB Infusion and Associated Compensated Metabolic Acidosis

***Nutrient Intake and Digestibility.*** Ketosis is generally associated with reduced feed intake (e.g., Benedet et al., 2019). This is likely related to the stimulation of oxidative metabolism and signals of energy status and satiety in the brain by ketones (Laeger et al., 2010) as well as to changes in acid-base balance (Zimpel et al., 2018). Abomasal infusion of BHB did not affect voluntary DMI of the TMR in the current study, likely because only a compensated metabolic acidosis was observed and because plasma BHB concentration, although elevated, may not have reached critical levels affecting satiety. In agreement, Zarrin et al. (2013, 2014) found no reduction in DMI of dairy cows when BHB was intravenously infused for 48 and 56 h, respectively. The decreased digestible NDF intake following abomasal infusion of BHB appears to be the result of a numerical decrease in TMR DMI and ATTD of NDF. The decrease in ATTD of CP and fat might be related to the increased intestinal permeability (suggested by the increased Co concentration in blood) upon abomasal infusion of BHB. An increased permeability may negatively affect hindgut fermentation and the stability of the intestinal microbiota. Taking this into account, it is unclear why the ATTD of starch increased with abomasal infusion of BHB when combined with 3.0 kg of corn starch/d, but not when combined with the lower infusion level of corn starch.

***Energy Partitioning.*** Abomasal infusion of BHB decreased blood glucose and plasma insulin concentra-

tions. This suggests that insulin did not play a role in the inhibiting effect of BHB on glucose concentrations, which is in agreement with studies in sheep (Schlumbohm and Harmeyer 2003) and dairy cows (Zarrin et al., 2013). Alongside insulin, gluconeogenesis is also hormonally regulated by glucagon (Zarrin et al., 2013). Glucagon increases plasma glucose concentration through stimulation of gluconeogenesis and glycogenolysis (Aronoff et al., 2004). Ketone bodies and NEFA can suppress glucagon secretion (Gerich et al., 1974), and Zarrin et al. (2013) reported decreased glucagon concentrations when BHB was intravenously infused in dairy cattle. Although not measured in the current study, glucagon secretion may have been depressed by the elevated plasma BHB concentration upon abomasal infusion of BHB, which may have resulted in less stimulation of gluconeogenesis or glycogenolysis and subsequently reduced blood glucose concentrations.

The effects of elevated plasma BHB concentrations on milk yield in literature are controversial. Several studies observed a decrease of daily milk production between 1% and 18% (e.g., Duffield et al., 2009; McArt et al., 2012), whereas others reported an increase of daily milk yield between 5% and 11% (e.g., Vanholder et al., 2015; Ruoff et al., 2017; reviewed by Benedet et al., 2019) in cows with an elevated plasma BHB concentration (>1.2 mmol/L). Ketone bodies can have a glucose-sparing effect in tissues where ketone bodies can be used as an energy source (Zarrin et al., 2013), but glucose is required as a unique source for mammary lactose synthesis (Zarrin et al., 2014). Despite the decreased blood glucose concentrations observed in the present study, milk yield and lactose yield were not affected by abomasal BHB infusion. This is in agreement with Zarrin et al. (2013; only milk yield reported) as well as Herrick et al. (2018). As demonstrated by Mebane and Madison (1962) and Madison et al. (1964), if the increased plasma BHB concentration in response to BHB infusion inhibited nonmammary peripheral glucose utilization, sufficient glucose may have been available to support milk production in the current study.

It was expected that abomasal infusion of BHB would result in an increased milk fat concentration, because BHB can be used as precursor for milk fat synthesis (Bauman and Griinari, 2003), and because increased milk fat concentrations are often observed in ketotic cows (e.g., Duffield et al., 2009; Vanholder et al., 2015; Benedet et al., 2019). However, an increase in milk fat concentration was not observed in the present study, perhaps because the increase in circulating BHB concentration was below threshold levels characteristic of ketotic dairy cows.

We observed an increase in energy retained as body fat in the present study upon abomasal infusion of BHB, suggesting that more body fat was accreted with BHB infusion. This may simply be due to the numerically increased GEI and decreased methane production upon BHB infusion, resulting in a significantly increased MEI. Despite increase MEI, milk energy output was unaffected by BHB infusion, leading to more energy being available for body accretion.

***Nitrogen Partitioning.*** Abomasal BHB infusion had minimal effects on N partitioning. Interestingly, milk urea concentration decreased in response to BHB infusion. This was also observed by Santschi et al. (2016), where milk urea N concentration was lower for ketotic cows compared with nonketotic cows, and this response was assigned to a lower protein intake for the cows with ketosis. In the present study, N intake decreased numerically and ATTD of N decreased significantly upon abomasal infusion of BHB. Hence, it is likely that less N was absorbed from the gastrointestinal tract, resulting in a smaller surplus (indicated as urea in milk or blood).

***Immune Response.*** The increased molar proportion of fecal butyrate upon BHB infusion might be related to the observed increased intestinal permeability, where elevated colonic butyrate levels are thought to have the ability to support a healthy gut environment (Brouns et al., 2002). Assuming that starch fermentation in the hindgut results in increased levels of butyrate (e.g., Mao et al., 2012), BHB infusion may also have caused a shift from starch digestion in small intestines to starch fermentation in hindgut.

Increased intestinal permeability (suggested by the increased Co concentration in blood) appears not to be related to the potential hyperosmolality that could have been caused by infusion of BHB as a ketone salt (Kameda et al., 1968). This is because the non-BHB treatments were formulated to contain the same amount of Na compared with the BHB treatments (Table 2). Although, increased permeability does appear to be attributable to BHB, it is unclear why the epithelium of the large intestine became more permeable or susceptible to damage upon BHB infusion.

## CONCLUSIONS

Fecal pH decreased with each level of corn starch infused into the abomasum (pH of 6.49, 6.00, and 5.15 with 0.0, 1.5, and 3.0 kg of corn starch infused/d, respectively), suggesting hindgut acidosis was induced when corn starch was abomasally infused. Abomasal infusion of BHB resulted in a compensated metabolic acidosis, which was characterized by a disturbance of the acid-base status (i.e., decreased blood total $CO_2$, $HCO_3$, and base excess, and trend for decreased urinary pH), whereas blood pH remained within a physiologi-

cally normal range. Overall, in this short-term experiment, induced hindgut acidosis was not associated with negative effects on milk production and composition, energy balance, or inflammatory response, and was positively associated with milk N efficiency. Similarly, the induced compensated metabolic acidosis was not associated with negative effects on milk production and composition or inflammatory response, and was being positively associated with energy retained as body fat. Long-term studies are needed to confirm these short-term findings.

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