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Abomasal infusion of ground corn and ammonium chloride in early-lactating Holstein-Friesian dairy cows to induce hindgut and metabolic acidosis

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ABSTRACT

Next to rumen acidosis, other forms of acidosis may also affect lactational performance of cows. Therefore, the effects of hindgut acidosis, induced via abomasal infusion of ground corn, and metabolic acidosis, induced via abomasal infusion of NH_4Cl, were studied in cows in early lactation. Observations were made on intake and digestibility of nutrients, lactation performance, energy and N partitioning, blood acid-base status, and rumen and hindgut fermentation characteristics. In a 6 × 6 Latin square design, 6 rumen-fistulated, second-lactation Holstein-Friesian dairy cows (48 ± 17 d in milk) were subjected to 5 d of continuous abomasal infusions of water as control, or solutions of 2.5 mol of NH_4Cl/d, 5.0 mol of NH_4Cl/d, 3.0 kg of ground corn/d, or the combination of ground corn with either of the 2 NH_4Cl levels, followed by 2 d of rest. Treatment solutions were administered via peristaltic pumps through infusion lines attached to the rumen cannula plug and an abomasal infusion line with a flexible disk (equipped with holes to allow digesta passage) to secure its placement through the sulcus omasi. A total mixed ration consisting of 70% grass silage and 30% concentrate (on dry matter basis) was fed at 95% of ad libitum intake of individual cows. The experiment was conducted in climate respiration chambers to determine feed intake, lactation performance, and energy and N balance. Abomasal infusion of NH_4Cl affected the acid-base status of the cows, but more strongly when in combination with abomasal infusion of ground corn. Metabolic acidosis (defined as a blood pH < 7.40, blood HCO_3 concentration < 25.0 mmol/L, and a negative base excess) was observed with 5.0 mol of NH_4Cl/d, 3.0 kg of ground corn/d + 2.5 mol of NH_4Cl/d, and 3.0 kg of ground corn/d + 5.0 mol of NH_4Cl/d. Metabolic acidosis was associated with decreased milk lactose content, metabolic body weight, energy retained as protein, and fecal N excretion, and increased urine N excretion, and tended to decrease intake of nutrients. Digestibility of several nutrients increased with 5.0 mol of NH_4Cl/d, likely as a result of decreased intake. Abomasal ground corn infusion resulted in hindgut acidosis, where fecal pH decreased from 6.86 without ground corn to 6.00 with ground corn, regardless of NH_4Cl level. The decrease in fecal pH was likely the result of increased hindgut fermentation, evidenced by increased fecal volatile fatty acid concentrations. Hindgut acidosis was associated with decreased digestibility of nutrients, except for starch, which increased, and crude fat, which was not affected. No systemic inflammatory response was observed, suggesting that the hindgut epithelium was not severely affected by the more acidic conditions or barrier damage. Abomasal infusion of ground corn increased milk yield, milk protein and lactose yield, fecal N excretion, N use efficiency, and total energy retained as well as energy retained in fat, and reduced milk fat content and urine N excretion.

Key words: dairy cow, early lactation, metabolic acidosis, hindgut acidosis

INTRODUCTION

In dairy cattle, several forms of acidosis related to nutrition can be distinguished. Rumen acidosis has been reviewed extensively, whereas the roles of hindgut and metabolic acidosis have received less attention until recently (Gressley et al., 2011; Plaizier et al., 2018). High-yielding dairy cows commonly suffer from production-limiting diseases, including milk fever and ketosis (Mulligan and Doherty, 2008). Many of these diseases interact, and it has only recently been recognized that prevention of these diseases is closely linked with addressing the gastrointestinal health of dairy cows (e.g., Enemark, 2008; Mulligan and Doherty, 2008). Risks of excessive hindgut fermentation and associated organic acid production increase with dietary fermentable carbohydrate content (starch content in particular) and with feed intake level (associated with reduced retention time of feed in the rumen; Gressley

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et al., 2011). Most of the VFA produced in the hindgut are absorbed through the intestinal epithelium (Argenzio et al., 1975; Gressley et al., 2011), but excessive fermentation in the hindgut can lead to an accumulation of VFA and other acids (Gressley et al., 2011) and consequently a decrease in digesta pH. The hindgut is less capable than the rumen of maintaining a stable digesta pH, due to the lack of bicarbonate from salivary secretion, absence of protozoa, and differences in the gut epithelium structure (Immig, 1996; Gressley et al., 2011; Dijkstra et al., 2012). A decrease in digesta pH due to hindgut fermentation may change the composition and functioning of hindgut microbial populations, may damage the gut epithelium, and consequently may affect both animal production and health (Gressley et al., 2011).

In addition to hindgut acidosis, cows in early lactation are at risk of developing metabolic acidosis associated with dietary factors, or as a consequence of rumen and hindgut acidosis (Enemark et al., 2002). Blood plasma pH is determined by several factors, including partial pressure of CO_2, concentration of nonvolatile weak buffers such as albumins and globulins, and strong ion difference (the difference between strong cations and anions; Stewart, 1983). The latter is of particular relevance in manipulation of DCAD [defined as mEq of $(Na^+ + K^+) - (Cl^- + S^{2-})$ per kg of DM; Afzaal et al., 2004] and its role in preventing hypocalcemia in the periparturient period (Charbonneau et al., 2006). With increased consideration of DCAD in dry-cow nutrition, interest has also grown in the effects of DCAD on lactating dairy cows. Some studies have suggested that there could be an optimal DCAD for maximizing feed consumption and milk yield. For example, Constable et al. (2017) suggested that the optimal DCAD is approximately +400 mEq/kg of DM for cows in early lactation and between +275 to +400 mEq/kg of DM for cows in mid-lactation. Hu and Murphy (2004) observed in a meta-analysis that DMI and milk yield increased quadratically with DCAD up to +400 mEq/kg of DM and +340 mEq/kg of DM, respectively. The response in milk yield is most likely related to increased energy and nutrient supply to support milk. Reduced palatability of the diet and induced metabolic acidosis due to lower cation-to-anion ratio have been proposed as the main reasons for reduced DMI (Zimpel et al., 2018). Apart from dietary change in DCAD to prevent hypocalcemia, dietary manipulation of DCAD can be seen as an experimental model that enables studying the consequences of metabolic acidosis.

Limited research has been performed in which experimentally induced hindgut acidosis and metabolic acidosis were studied simultaneously in early-lactation dairy cows. This is of great interest, because a relation may exist between acidosis in the hindgut and the occurrence of metabolic acidosis, especially when acidosis in the hindgut is accompanied by metabolic consequences, affecting acid-base status of the cow, and vice versa. The aims of the current study were (1) to induce hindgut and metabolic acidosis via abomasal infusion of ground corn and NH_4Cl, respectively, and (2) to determine the effects of these physiological states on feed intake, apparent total-tract digestibility (**ATTD**) of nutrients, energy and N partitioning, lactation performance, blood acid-base status, respiratory and nutrient status, and rumen and hindgut fermentation characteristics.

We hypothesized that abomasally infused ground corn would be fermented in the hindgut, produce VFA, and subsequently decrease digesta pH, resulting in hindgut acidosis. Abomasal infusion of NH_4Cl was expected to result in metabolic acidosis, where the absorption of Cl from the gastrointestinal tract would reduce the strong ion difference in blood and decrease blood pH. When both ground corn and NH_4Cl were infused into the abomasum, we expected to induce both hindgut and metabolic acidosis, with metabolic acidosis being more severe compared with only NH_4Cl 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 2018 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 approved by the Central Committee of Animal Experiments (The Hague, the Netherlands; 2018.D-0013.001).

Six rumen-fistulated, second-lactation Holstein-Friesian dairy cows with an average milk production (±SD) of 34.2 ± 2.87 kg/d at 48 ± 17 DIM at the start of the experiment were randomly assigned to a 6 × 6 Latin square design with 6 treatments. Each experimental period (n = 6) consisted of 120 h of continuous abomasal infusion followed by 48 h of rest (Figure 1). 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**).

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Diet and Feeding

Cows were fed a TMR consisting of 70% grass silage and 30% concentrate on a DM basis (Table 1). To determine the ATTD of nutrients, TiO_2 was included in the concentrate (5 g/kg of DM) as an external marker. The concentrate was in meal form and produced by Research Diet Services (RDS BV, Wijk bij Duurstede, the Netherlands) in a single batch. The diet was formulated to meet 98 and 91% of NE_L (VEM; Dutch feed unit lactation; 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 35 kg/d of milk containing 4.0% fat and 3.5% protein.

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 5% 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. Fresh feed was allocated twice daily at 0500 and 1530 h by manually mixing the roughage and concentrate portions into a TMR for individual cows. The roughage portion (i.e., grass silage + water) of the diet was mixed 3 times weekly using a self-propelled mixer wagon (Strautmann Verti-Mix 500, Bad Laer, Germany) equipped with a cutter loader system and an electronic weighing scale, and 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. Before providing fresh feed, feed refusals were collected and weighed to determine daily feed intake. For an 85.5-h period over d 2 to d 6 of each experimental period (1530 h on d 2 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 that will be described. Cows had individual and free access to clean drinking water throughout the entire experiment.

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

Item	Grass silage[2]	Concentrate[3]	TMR
DM (g/kg of product)	549	891	447
OM	900	917	905
CP	171	181	174
Crude fat	37	94	54
GE[4] (MJ/kg of DM)	19.0	19.3	19.1
NDF	508	318	451
ADF	284	192	256
ADL	13	19	15
Starch	—[5]	150	45
Sugar	98	71	90
NE_L[6] (MJ/kg of DM)	6.2	8.5	6.9
DVE[7]	66	157	93
OEB[8]	37	−16	21
Sodium	4.6	2.7	4.0
Potassium	30.9	12.0	25.2
Chloride	19.5	4.0	14.9
Sulfur	4.1	1.9	3.4

[1]The TMR was composed of 57% grass silage, 15% concentrate, and 28% water on product basis, which was equal to 700 g/kg of DM grass silage and 300 g/kg of DM concentrate.

[2]Ensiling characteristics: acetic acid = 7 g/kg of DM, lactic acid = 11 g/kg of DM, ammonia-N = 6% total N, and pH = 5.6. DCAD = 184 mEq/kg of DM.

[3]Concentrate contained (g/kg of DM): wheat = 200, soybean hulls = 200, rumen-protected soybean meal (Rumi-S, NuScience, Utrecht, the Netherlands) = 175, sugar beet pulp = 150, palm kernel flakes = 100, palm oil = 50, molasses = 40, linseed = 28, rumen-protected rapeseed meal (Mervobest, NuScience) = 25, CaCO3 = 14, trace mineral and vitamin mix = 8.0, NaCl = 5, MgO = 0.5, and TiO2 = 5.0. DCAD = 152 mEq/kg of DM.

[4]GE = gross energy.

[5]Not determined.

[6]Dutch feed unit lactation (van Es, 1978).

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

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

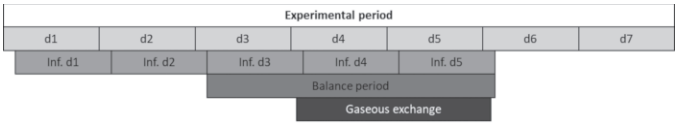

Figure 1. Design of a single 7-d experimental period. Inf. d1 to Inf. d5 = 120-h abomasal infusion period beginning at 0900 h on d 1 and ending at 0900 h on d 6 of each experimental period. The infusion period was followed by a 48-h washout period. Balance period = 71-h period of total manure collection with milk and feces samples from 1000 h on d 3 until 0900 h on d 6. Gaseous exchange = 48-h period of gas production and consumption measured from 0800 h on d 4 until 0800 h on d 6.

Abomasal Infusions

Infusion lines were placed in the abomasum via the rumen cannula 2 d before the first experimental period and were checked twice daily for patency and position. 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., Parma, ID). For the treatments containing no ground corn (see subsequent description), the infusion lines (Tygon S3 E-3603, 3.2 mm internal diameter, 6.4 mm outer diameter; VWR, Amsterdam, the Netherlands) were connected between the treatment solutions and the multichannel peristaltic

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pumps with luer-to-tubing connectors (Sigma-Aldrich, St. Louis, MO) and subsequently inserted and secured into the braided polyvinyl chloride hose feeding into the rumen cannula. For the treatments containing ground corn (see subsequent description), infusion lines with a larger diameter (Tygon S3 E-3603, 4.8 mm internal diameter, 8.0 mm outer diameter; VWR) were used and connected in the same way as previously described for the treatments without ground corn. Treatments containing ground corn were maintained in suspension by continuous stirring.

Infusion treatments were (1) no ground corn, no NH_4Cl (DCAD value basal TMR + infusion of 184 mEq/kg of DM); (2) no ground corn, 2.5 mol of NH_4Cl/d (DCAD value basal TMR + infusion of 60 mEq/kg of DM); (3) no ground corn, 5.0 mol of NH_4Cl/d (DCAD value basal TMR + infusion of −65 mEq/kg of DM); (4) 3.0 kg of ground corn/d, no NH_4Cl (DCAD value basal TMR + infusion of 212 mEq/kg of DM); (5) 3.0 kg of ground corn/d + 2.5 mol of NH_4Cl/d (DCAD value basal TMR + infusion of 99 mEq/kg of DM); and (6) 3.0 kg of ground corn/d + 5.0 mol of NH_4Cl/d (DCAD value basal TMR + infusion of −10 mEq/kg of DM). The DCAD value was calculated based on the equation described by CVB (2018) and the reported Na, K, Cl, and S values for each separate dietary ingredient. The treatments were administered in 20-L batches that were replenished daily and infused at a rate ranging from 841 to 969 g/h (Table 2) to facilitate 120 h of continuous infusion (0900 h on d 1 until 0900 h on d 6 of each experimental period; Figure 1). Ground corn was chosen as the source to induce hindgut acidosis for 2 reasons. First, in common practice, ground corn is one of the major starch sources fed to dairy cattle in early lactation. Second, ground corn supplies more rumen-resistant starch flowing into the intestine compared with wheat and barley. The ground corn was manipulated (performed by Heme N.V., Waddinxveen, the Netherlands) to represent the rumen-resistant fraction of ground corn. First, the ground corn (Cargill, Velddriel, the Netherlands) was sieved over a 1-mm screen. The fine fraction was discarded, because this fraction is assumed to be largely rumen-fermentable. Second, the remaining coarse fraction was ground to pass a 1-mm screen and again sieved over a 0.63-mm screen. After this second step of sieving, the remaining coarse fraction was discarded, and only the fine fraction was used for the infusion treatments. The last 2 steps (i.e., sieving + discarding the coarse fraction) were performed to minimize obstruction in the infusion lines as well as to aid in keeping the infusion treatment in solution. The differences in DCAD values were achieved by manipulation of dietary Cl via the addition of different amounts of NH_4Cl to the infusion treatments (Table 2).

Table 2. Abomasal infusion treatments for early-lactation dairy cows; target and realized

Treatment	Target (g/d, unless stated otherwise)						Realized[1] (g/d, unless stated otherwise)			
	NH_4Cl	Ground corn[2]	Urea	WPI[3]	Water (L/d)	Infusion rate (g/h)	Success rate (%)	NH_4Cl	Ground corn	DCAD (mEq/kg of DM)[4]
0.0 mol of NH_4Cl/d	0.0	0	150	25.7	20	841	95.1	0.0	0	184
2.5 mol of NH_4Cl/d	133.8	0	75	25.7	20	843	99.0	132.4	0	60
5.0 mol of NH_4Cl/d	267.5	0	0	25.7	20	846	100.0	267.6	0	−65
3.0 kg of ground corn/d + 0.0 mol of NH_4Cl/d	0.0	3,000	150	0.0	20	965	92.8	0.0	2,785	212
3.0 kg of ground corn/d + 2.5 mol of NH_4Cl/d	133.8	3,000	75	0.0	20	967	90.1	120.5	2,703	99
3.0 kg of ground corn/d + 5.0 mol of NH_4Cl/d	267.5	3,000	0	0.0	20	969	87.2	233.2	2,615	−10

[1]Averaged over the 6 experimental periods.
[2]Chemical composition (in g/kg of DM, unless stated otherwise): DM = 883 g/kg, OM = 984, CP = 91, crude fat = 51, gross energy (GE) = 18.9 MJ/kg of DM, NDF = 91, ADF = 27, ADL = 0, starch = 686, and sugar = 25.
[3]Whey protein isolate; Pure Whey Isolate 97, Bulk Powders, Colchester, UK. Chemical composition (in g/kg of DM, unless stated otherwise): DM = 946 g/kg, OM = 980, CP = 976, crude fat = 4, GE = 23.0 MJ/kg of DM.
[4]Based on TMR composition (DCAD value of individual ingredients on g/kg of DM basis) + realized abomasal infusion of the treatments.

Climate Respiration Chambers

The cows were housed in CRC for the experimental periods to determine gaseous exchange, energy and N balance, and ATTD of nutrients. Detailed descriptions of the CRC design and gas measurements are reported by Heetkamp et al. (2015) and van Gastelen et al. (2015). Briefly, the relative humidity and temperature in each CRC compartment (area = 11.8 m^2, volume = 34.5 m^3) were maintained at 80% and 10°C, respectively. The CRC were equipped with thin walls with windows to allow audio-visual contact between cows, to minimize the effects of social isolation on cow behavior and performance. 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. Calibration gases were sampled once daily for gas analysis instead of the inlet air. 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. Before the cows entered the CRC and after the final experimental period, 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 99.7% (ranging between 98.5 and 101.7% 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 was 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 weight was recorded at each milking. Milk samples were collected at each milking during the balance period (6 milkings; Figure 1) 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 (5 g/kg of milk) was collected at each milking during the balance period (6 milkings), and stored at −20°C until energy and N analyses.

Samples of rumen fluid, feces, and urine were collected immediately before each milking event during the balance period (n = 6). Rumen fluid samples (~200 mL) were obtained as described by van Zijderveld et al. (2011), and were composed of equal volumes collected from the front and middle of the ventral sac and from the cranial sac of the rumen. Fecal samples were collected by rectal grab sampling. Urine samples were collected by stimulating the dairy cows to urinate by rubbing the perineum using a vertical movement. After collection, feces, urine, and rumen fluid were thoroughly mixed, and their pH was immediately measured using an electronic pH meter (HI9024C, Hanna Instruments, IJsselstein, the Netherlands). Urine samples were subsequently discarded. Subsamples of feces (~100 g) and rumen fluid (600 μL) were collected and acidified with an equal volume of orthophosphoric acid (0.85 mg/L) containing 19.68 m*M* isocaproic acid as internal standard. Samples were subsequently frozen (−20°C) until VFA analysis.

Samples of grass silage and the concentrate were collected 3 times weekly during feed preparation. These samples were subsequently pooled per experimental period and stored at −20°C until analysis. During balance periods in the CRC, feed residues were collected twice daily (0500 h and 1530 h), weighed, and stored at 4°C. At the end of each experimental period, 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 (gaseous exchange; Figure 1), whereas energy and N balance and the ATTD of nutrients were based on manure and feces collection from d 3 (1000 h) through d 6 (0900 h; balance period; Figure 1). The end of the balance period corresponded with the end of the 120-h infusion period. 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 fresh total collection period. The feces and urine produced during the balance period were quantitatively collected as manure (by cleaning each CRC compartment), weighed, mixed, subsampled, and stored at −20°C until analysis. To quantify contribution of N from volatilized ammonia appearing from excreted and mixed feces and urine, samples were collected of condensed water from the chamber heat exchanger of each CRC compartment and from a 25% sulfuric acid solution (wt/wt) in which aerial ammonia in outflowing air was trapped. These

samples were stored at 4°C until analysis. During the balance period, rectal grab samples of feces (~300 g) were collected twice daily at 0500 h and 1530 h (6 samples in total) and immediately pooled into a composite sample by cow, which was stored at −20°C until analysis.

On d 4 and 5 of each experimental period, blood samples were collected from the coccygeal vessels into 10-mL sodium heparin and potassium EDTA vacutainers (Becton Dickinson, Rutherford, NJ) at 0800, 1000, 1200, and 1400 h. At each sampling moment, a subsample was collected immediately from the heparin vacutainer and analyzed by a hand-held VetScan i-STAT 1 analyzer (Abaxis Inc., Union City, CA) using disposable CG8+ cartridges (Abbott, Princeton, NJ). Immediately thereafter, both the heparin and potassium EDTA vacutainers were placed in ice and centrifuged at 5,000 × *g* for 15 min at room temperature, aliquoted, and stored at −20°C until further analysis (i.e., each time point was analyzed separately).

Analytical Procedures

Samples of grass silage, concentrate, feed residues, 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 (Peppink 100AN, Olst, the Netherlands) and an ultra-centrifugal mill for all other samples (Retsch ZM200, Retsch GmbH, Haan, Germany). Wet chemical analyses 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 were 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, Heitersheim, Germany) was used to determine gross energy (**GE**) content. Crude protein was calculated as N × 6.25.

Grass silage and the concentrate were analyzed for DM, ash, N, crude fat, starch (concentrate only), sugars, NDF, ADF, ADL, GE, and Ti (concentrate only). Feed residues were analyzed for DM. Manure samples were analyzed for DM, N, and GE. Feces 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, BHB, and acetone content by mid-infrared spectroscopy (ISO 9622; International Organization for Standardization, 2013; VVB, Doetinchem, the Netherlands). Pooled milk samples were analyzed for GE and N in fresh material as previously described.

For determination of VFA, the rumen fluid samples and fecal samples were analyzed as described by van Gastelen et al. (2015). Briefly, samples were thawed and centrifuged for 5 min at 14,000 × *g* at room temperature. The clear supernatant (1 μL) was injected onto a gas chromatograph (Fisons HRGC Mega 2, CE Instruments, Milan, Italy) with a split/splitless injector operated in split mode (split ratio 1:10), at a temperature of 225°C, using a capillary column (EC-1000, Alltech, Deerfield, IL; 30 m, 0.53 mm internal diameter, 1 μm film thickness) and helium as carrier gas, and fitted to a flame ionization detector. Identification and quantification were conducted with a chemical standard solution (0.85 mg/L orthophosphoric acid), including an internal standard (19.681 m*M* isocaproic acid) for correction.

Blood plasma was analyzed by the Utrecht University Veterinary Diagnostic Laboratory (Utrecht, the Netherlands) for urea, insulin, triglycerides, BHB, non-esterified fatty acids (**NEFA**), IGF-1, albumin, serum amyloid A (**SAA**), and haptoglobin. Plasma NEFA, BHB, insulin, and urea were analyzed as described by van Knegsel et al. (2007). Plasma IGF-1 (cat. no. L2KIGF, Siemens Healthineers, Erlangen, Germany) and SAA (Phase SAA assay, cat. no. TP-802, Tridelta Development Limited, Maynooth, Ireland) were analyzed using the advanced immunoassay Immulite 2000 system (Siemens, The Hague, the Netherlands). Plasma haptoglobin (Phase Haptoglobin Assay, cat. no. TP-801, Tridelta Development Limited), albumin (cat. no. OSR6102, Beckman Coulter Inc., Brea, CA), and triglycerides (cat. no. OSR60118, Beckman Coulter Inc.) were analyzed using the clinical chemistry analyzer Olympus AU-860 (Beckman Coulter Inc.).

Calculations and Statistical Analysis

Reported values for nutrient content of the TMR were calculated from ration composition and analyzed values obtained for the grass silage and concentrate. The NE_L was calculated with the VEM (Dutch feed unit lactation) system according to Van Es (1978). For the grass silage, digestible protein, rumen-degradable protein balance (see Table 1), and NE_L were calculated based on the chemical composition as obtained by near-infrared spectroscopy analysis (Eurofins Agro, Wageningen, the Netherlands). For the concentrate, digestible protein, rumen-degradable protein, and NE_L were calculated based on table values for composition of the ingredients (CVB, 2018).

Fat- and protein-corrected milk yield (FPCM; kg/d) was calculated as (0.337 + 0.116 × fat % + 0.06 × protein %) × milk yield (CVB, 2018). Heat production (kJ/d) was calculated as 16.175 × VO_2 (L/d) + 5.021

× VCO_2 (L/d), where VO_2 and VCO_2 are volumes of O_2 consumed and CO_2 produced, respectively (Gerrits et al., 2015). The ATTD of nutrients was calculated taking into account nutrient inflow with the TMR as well as the abomasal 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 VFA and pH of rumen fluid, feces, and urine (pH only) were averaged over the analyzed sampling times. The variables related to energy and N balance were expressed per kilogram of metabolic BW 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., Cary, NC). The model contained main effects and interaction effects of infusion treatment factors (NH_4Cl and ground corn) 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 NH_4Cl effect or ground corn × NH_4Cl interaction [i.e., between 2 levels of ground corn (0.0 or 3.0 kg/d) and 3 levels of NH_4Cl (0.0, 2.5, or 5.0 mol/d)] 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 in each square. We assessed for carryover effects between periods by testing for an effect of the previous treatment in the ANOVA.

RESULTS

Nutrient Intake and Digestibility

Nutrient intake and ATTD of nutrients were not affected by ground corn × NH_4Cl interactions (Table 3). Infusion of ground corn increased ($P \leq 0.025$) the total intake (TMR + infusion) of DM, OM, fat, starch, and GE, and tended to increase the intake of digestible OM ($P = 0.064$), relative to no infusion of ground corn. Ground corn infusion decreased ($P \leq 0.002$) the ATTD of DM, OM, CP, GE, and NDF, and increased ($P < 0.001$) the ATTD of starch compared with no infusion of ground corn. Regardless of ground corn inclusion, 5.0 mol of NH_4Cl/d decreased ($P \leq 0.048$) the total intake of CP, digestible NDF, and starch relative to no NH_4Cl infusion, with no difference observed between 0.0 and 2.5 mol of NH_4Cl/d and between 2.5 and 5.0 mol of NH_4Cl/d. Compared with no NH_4Cl infusion, both 2.5 and 5.0 mol of NH_4Cl/d tended ($P \leq 0.094$) to decrease the total intake of OM, digestible OM, crude fat, GE, NDF, ADF, ADL, and sugar. Infusion of 5.0 mol of NH_4Cl/d increased ($P = 0.009$) the ATTD of CP relative to both 0.0 and 2.5 mol of NH_4Cl/d, with no difference between 0.0 and 2.5 mol of NH_4Cl/d. Additionally, 5.0 mol of NH_4Cl/d increased ($P < 0.029$) the ATTD of fat and starch relative to no NH_4Cl infusion, with no difference between 0.0 and 2.5 mol of NH_4Cl/d and between 2.5 and 5.0 mol of NH_4Cl/d.

Lactation Performance

Only milk lactose content tended to be affected by a ground corn × NH_4Cl interaction (Table 4), where lactose content decreased as NH_4Cl increased from 0.0 to 5.0 mol/d, but the decrease tended to be less when ground corn was included in the infusion ($P = 0.075$). Infusion of ground corn increased ($P \leq 0.041$) milk yield, lactose content and yield, and protein yield, tended to increase ($P = 0.093$) fat- and protein-corrected milk yield, and decreased ($P \leq 0.001$) content of fat, BHB, and acetone relative to no infusion of ground corn. Regardless of ground corn inclusion, milk lactose content decreased ($P < 0.001$) with increasing level of NH_4Cl infusion. No other lactation performance variable was affected by NH_4Cl.

Blood Constituents

Concerning the i-STAT measurements, whole-blood pH, base excess, HCO_3, total CO_2, and ionized calcium were affected by ground corn × NH_4Cl interactions ($P \leq 0.037$; Table 5). In the absence of ground corn, blood pH, base excess, HCO_3, and total CO_2 were lower for 5.0 mol of NH_4Cl/d compared with 0.0 and 2.5 mol of NH_4Cl/d, with no difference in these blood constituents between 0.0 and 2.5 mol of NH_4Cl/d. By contrast, in the presence of ground corn, blood pH, base excess, HCO_3, and total CO_2 were lower with both 2.5 and 5.0 mol of NH_4Cl/d compared with no NH_4Cl infusion, with no difference between 2.5 and 5.0 mol of NH_4Cl/d. For ionized calcium the same pattern of treatment effects was observed, but in the opposite direction. In the absence of ground corn, ionized calcium was higher with 5.0 mol of NH_4Cl/d compared with both 0.0 and 2.5 mol of NH_4Cl/d, and in presence of ground corn ionized calcium was higher for both 2.5 and 5.0 mol of NH_4Cl/d compared with no NH_4Cl infusion (with no difference between 2.5 and 5.0 mol of NH_4Cl/d). Infusion of ground corn increased ($P \leq 0.011$) whole-blood concentration of sodium and glucose relative to no ground corn infusion. Regardless of ground corn inclusion, increasing infusion of NH_4Cl decreased ($P < 0.001$) partial pressure of CO_2. Additionally, 2.5 mol of NH_4Cl/d increased ($P = 0.019$) blood potassium con-

Table 3. Nutrient intake (TMR + abomasal infusion) and apparent total-tract digestibility of nutrients of early-lactation dairy cows abomasally infused with ground corn and NH_4Cl

	Ground corn (kg/d)		NH_4Cl (mol/d)				*P*-value		
Item	0.0	3.0	0.0	2.5	5.0	SEM	Ground corn	NH_4Cl	Ground corn × NH_4Cl[1]
Nutrient intake (TMR + abomasal infusions, kg/d unless stated otherwise)									
DM	13.0	14.5	14.4	14.0	12.8	0.60	0.025	0.103	0.371
OM	11.7	13.2	13.2	12.6	11.4	0.55	0.012	0.055	0.366
Digestible OM	8.4	9.2	9.3	8.9	8.2	0.39	0.064	0.076	0.230
CP	2.71	2.71	2.86[a]	2.75[ab]	2.52[b]	0.119	0.960	0.048	0.432
Crude fat	0.70	0.78	0.78	0.75	0.68	0.031	0.023	0.067	0.316
Gross energy (MJ/d)	245.2	273.4	273.6	263.8	240.6	11.51	0.021	0.078	0.362
NDF	5.77	5.60	6.01	5.79	5.26	0.270	0.537	0.094	0.354
Digestible NDF	4.19	3.90	4.29[a]	4.14[ab]	3.70[b]	0.199	0.121	0.041	0.203
ADF	3.28	3.13	3.38	3.27	2.96	0.159	0.316	0.086	0.299
ADL	0.19	0.18	0.19	0.19	0.17	0.010	0.165	0.079	0.248
Starch	0.58	2.18	1.44[a]	1.39[ab]	1.31[b]	0.038	<0.001	0.031	0.393
Sugar	1.14	1.11	1.19	1.16	1.02	0.058	0.601	0.052	0.248
Apparent digestibility (% of intake)									
DM	71.7	69.1	70.0	69.8	71.4	0.90	<0.001	0.123	0.633
OM	72.3	69.9	71.0	70.5	71.7	0.92	0.001	0.294	0.512
CP	68.5	62.8	64.4[a]	64.9[a]	67.6[b]	1.03	<0.001	0.009	0.453
Crude fat	70.4	70.4	68.9[a]	70.0[ab]	72.4[b]	1.47	0.967	0.006	0.551
Gross energy	70.2	67.3	68.2	68.2	69.7	0.80	<0.001	0.132	0.469
NDF	72.6	69.7	71.5	71.1	70.8	0.86	0.002	0.816	0.623
Starch	93.6	95.0	93.6[a]	94.4[ab]	94.9[b]	0.44	<0.001	0.029	0.466

[a,b]Values with different superscripts indicate a significant difference ($P < 0.05$) between 0.0, 2.5, and 5.0 mol of NH_4Cl/d.

[1]Interaction between 2 levels of ground corn (0.0 and 3.0 kg/d) and 3 levels of NH_4Cl (0.0, 2.5, and 5.0 mol/d).

tent relative to no NH_4Cl infusion, but with no difference observed between 0.0 and 5.0 mol of NH_4Cl/d and between 2.5 and 5.0 mol of NH_4Cl/d. Furthermore, 5.0 mol of NH_4Cl/d decreased ($P = 0.038$) blood glucose content relative to no NH_4Cl infusion, with no difference between 0.0 and 2.5 mol of NH_4Cl/d and between 2.5 and 5.0 mol of NH_4Cl/d. Infusion of NH_4Cl tended to decrease blood sodium content ($P = 0.065$).

Plasma Constituents

Concerning laboratory blood plasma analyses, concentration of plasma triglycerides was affected by a ground corn × NH_4Cl interaction ($P = 0.005$; Table 6). The effect of NH_4Cl became apparent only in the absence of ground corn, where triglyceride concentration was higher with 5.0 mol of NH_4Cl/d compared

Table 4. Milk production and composition of early-lactation dairy cows abomasally infused with ground corn and NH_4Cl

	Ground corn (kg/d)		NH_4Cl (mol/d)				*P*-value		
Item	0.0	3.0	0.0	2.5	5.0	SEM	Ground corn	NH_4Cl	Ground corn × NH_4Cl[1]
Milk yield (kg/d)	20.0	22.8	21.2	22.2	20.7	1.37	0.002	0.282	0.902
FPCM[2] (kg/d)	21.2	22.5	21.9	22.6	21.0	0.95	0.093	0.190	0.989
Feed efficiency (kg of FPCM/kg of DMI)	1.66	1.59	1.54	1.62	1.72	0.090	0.306	0.181	0.219
Fat content (g/100 g)	4.71	4.15	4.51	4.41	4.37	0.216	<0.001	0.428	0.540
Protein content (g/100 g)	3.02	2.99	3.02	3.02	2.98	0.123	0.307	0.636	0.843
Lactose content (g/100 g)	4.34	4.41	4.48[a]	4.38[b]	4.27[c]	0.106	0.041	<0.001	0.075
Fat yield (g/d)	936	928	944	963	890	41.4	0.805	0.184	0.913
Protein yield (g/d)	600	673	638	658	613	19.9	<0.001	0.170	0.993
Lactose yield (g/d)	871	1,000	952	972	882	59.7	0.001	0.110	0.996
BHB (mmol/L)	0.181	0.120	0.154	0.151	0.148	0.0143	<0.001	0.811	0.588
Acetone (mmol/L)	0.318	0.218	0.268	0.269	0.267	0.0203	<0.001	0.994	0.882

[a–c]Values with different superscripts indicate a significant difference ($P < 0.05$) between 0.0, 2.5, and 5.0 mol of NH_4Cl/d.

[1]Interaction between 2 levels of ground corn (0.0 and 3.0 kg/d) and 3 levels of NH_4Cl (0.0, 2.5, and 5.0 mol/d).

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

with 2.5 mol of NH_4Cl/d, with no difference between 0.0 and 2.5 mol of NH_4Cl/d and between 0.0 and 5.0 mol of NH_4Cl/d. Plasma albumin concentration tended ($P = 0.080$) to be affected by a ground corn × NH_4Cl interaction, where 2.5 mol of NH_4Cl/d resulted in a numerically higher concentration than 0.0 and 5.0 mol of NH_4C/d in the presence of ground corn, but a numerically lower concentration in the absence of ground corn. Ground corn infusion decreased ($P < 0.035$) plasma concentrations of urea, insulin, BHB, and NEFA, and increased ($P = 0.005$) IGF-1 relative to no ground corn infusion. Regardless of ground corn inclusion, 2.5 and 5.0 mol of NH_4Cl/d decreased ($P = 0.011$) plasma haptoglobin concentration compared with no NH_4Cl infusion, with no difference between 2.5 and 5.0 mol of NH_4Cl/d.

Energy and Nitrogen Balance

None of the variables related to energy or nitrogen balance were affected by ground corn × NH_4Cl interactions (Table 7). Infusion of ground corn increased ($P < 0.040$) the respiratory quotient, GE intake, energy excreted in manure, metabolizable energy intake (**MEI**), energy secreted in milk, total energy retention, and energy retained as fat, relative to no infusion of ground corn. Regardless of ground corn inclusion, 5.0 mol of NH_4Cl/d decreased ($P < 0.001$) metabolic BW relative to 0.0 and 2.5 mol of NH_4Cl/d, with no difference observed between the latter 2. Additionally, 5.0 mol of NH_4Cl/d decreased ($P \leq 0.015$) both CH_4 production and energy retained as protein relative to no NH_4Cl infusion, with no difference between 0.0 and 2.5 mol

Table 5. Whole-blood constituents, measured with a VetScan i-STAT 1 analyzer (Abaxis Inc., Union City, CA), of early-lactation dairy cows abomasally infused with ground corn and NH_4Cl

	Ground corn (kg/d)		NH_4Cl (mol/d)				*P*-value		
Item	0.0	3.0	0.0	2.5	5.0	SEM	Ground corn	NH_4Cl	Ground corn × NH_4Cl[1]
pH[2]	7.41	7.38	7.48	7.40	7.32	0.015	0.117	<0.001	0.037
pCO_2[3] (mm Hg)	42.1	42.4	46.1[a]	42.1[b]	38.6[c]	1.70	0.713	<0.001	0.080
pO_2[4] (mm Hg)	72.6	71.9	68.7	74.8	73.2	8.58	0.916	0.753	0.404
Base excess[5] (mmol/L)	2	1	9	1	−6	1.2	0.263	<0.001	0.013
HCO_3[6] (mmol/L)	27.3	26.1	33.7	26.4	20.0	1.10	0.253	<0.001	0.009
Total CO_2[7] (mmol/L)	29	27	35	28	21	1.2	0.288	<0.001	0.011
sO_2[8] (%)	79	80	79	78	80	3.7	0.760	0.917	0.411
Sodium (mmol/L)	138	139	138	138	137	0.7	0.009	0.065	0.300
Potassium (mmol/L)	4.7	4.7	4.6[a]	4.8[b]	4.7[ab]	0.05	0.554	0.019	0.255
Ionized calcium[9] (mmol/L)	1.29	1.29	1.22	1.28	1.35	0.014	0.963	<0.001	0.011
Glucose (mg/dL)	61	64	64[a]	63[ab]	61[b]	1.6	0.011	0.038	0.771
Hematocrit[10] (% PCV)	24	24	24	24	24	0.7	0.429	0.962	0.213
Hemoglobin (g/dL)	8.20	8.12	8.16	8.16	8.17	0.242	0.474	0.998	0.247

[a–c]Values with different superscripts indicate a significant difference ($P < 0.05$) between 0.0, 2.5, and 5.0 mol of NH_4Cl/d.

[1]Interaction between 2 levels of ground corn (0.0 or 3.0 kg/d) and 3 levels of NH_4Cl (0.0, 2.5, or 5.0 mol/d). If a significant ground corn × NH_4Cl interaction was found, a different superscript (k–n) in the footnote of that particular variable indicates a significant difference ($P < 0.05$).

[2]Ground corn × NH_4Cl interaction: 0.0 mol of NH_4Cl/d with 0.0 kg of starch/d = 7.50[k]; 2.5 mol of NH_4Cl/d with 0.0 kg of starch/d = 7.44[kl]; 5.0 mol of NH_4Cl/d with 0.0 kg of starch/d = 7.30[m]; 0.0 mol of NH_4Cl/d with 3.0 kg of starch/d = 7.46[k]; 2.5 mol of NH_4Cl/d with 3.0 kg of starch/d = 7.36[lm]; 5.0 mol of NH_4Cl/d with 3.0 kg of starch/d = 7.34[m].

[3]Partial pressure of CO_2.

[4]Partial pressure of O_2.

[5]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 mm Hg (Corey, 2003). Ground corn × NH_4Cl interaction: 0.0 mol of NH_4Cl/d with 0.0 kg of starch/d = 10[k]; 2.5 mol of NH_4Cl/d with 0.0 kg of starch/d = 5[k]; 5.0 mol of NH_4Cl/d with 0.0 kg of starch/d = −7[l]; 0.0 mol of NH_4Cl/d with 3.0 kg of starch/d = 9[k]; 2.5 mol of NH_4Cl/d with 3.0 kg of starch/d = −2[l]; 5.0 mol of NH_4Cl/d with 3.0 kg of starch/d = −4[l].

[6]Ground corn × NH_4Cl interaction: 0.0 mol of NH_4Cl/d with 0.0 kg of starch/d = 34.0[k]; 2.5 mol of NH_4Cl/d with 0.0 kg of starch/d = 29.4[k]; 5.0 mol of NH_4Cl/d with 0.0 kg of starch/d = 18.6[l]; 0.0 mol of NH_4Cl/d with 3.0 kg of starch/d = 33.5[k]; 2.5 mol of NH_4Cl/d with 3.0 kg of starch/d = 23.4[l]; 5.0 mol of NH_4Cl/d with 3.0 kg of starch/d = 21.3[l].

[7]Ground corn × NH_4Cl interaction: 0.0 mol of NH_4Cl/d with 0.0 kg of starch/d = 35[k]; 2.5 mol of NH_4Cl/d with 0.0 kg of starch/d = 30[k]; 5.0 mol of NH_4Cl/d with 0.0 kg of starch/d = 20[l]; 0.0 mol of NH_4Cl/d with 3.0 kg of starch/d = 35[k]; 2.5 mol of NH_4Cl/d with 3.0 kg of starch/d = 25[l]; 5.0 mol of NH_4Cl/d with 3.0 kg of starch/d = 23[l].

[8]O_2 saturation.

[9]Ground corn × NH_4Cl interaction: 0.0 mol of NH_4Cl/d with 0.0 kg of starch/d = 1.22[k]; 2.5 mol of NH_4Cl/d with 0.0 kg of starch/d = 1.26[km]; 5.0 mol of NH_4Cl/d with 0.0 kg of starch/d = 1.38[ln]; 0.0 mol of NH_4Cl/d with 3.0 kg of starch/d = 1.23[k]; 2.5 mol of NH_4Cl/d with 3.0 kg of starch/d = 1.31[mn]; 5.0 mol of NH_4Cl/d with 3.0 kg of starch/d = 1.32[mn].

[10]PCV = packed cell volume; the ratio between the volume of red blood cells and the total volume of blood.

of NH_4Cl/d and between 2.5 and 5.0 mol of NH_4Cl/d. Respiratory quotient and heat production tended to decrease with increasing level of NH_4Cl infusion ($P \leq 0.068$).

Infusion of ground corn increased ($P < 0.006$) N excreted in feces, N secreted in milk, and N efficiency, and decreased N excreted in urine and N collected in condensed water and air, relative to no ground corn infusion. Regardless of ground corn inclusion, 5.0 mol of NH_4Cl/d increased ($P = 0.002$) N excreted in urine relative to both 0.0 and 2.5 mol of NH_4Cl/d, with no difference between the latter 2. Infusion of 5.0 mol of NH_4Cl/d decreased ($P \leq 0.042$) N excreted in feces and N balance relative to no NH_4Cl infusion, with no difference observed between 0.0 and 2.5 mol of NH_4Cl/d and between 2.5 and 5.0 mol of NH_4Cl/d. Nitrogen efficiency tended ($P = 0.058$) to increase with increasing infusion level of NH_4Cl infusion.

Rumen Fluid, Feces, and Urine

The pH of rumen fluid, feces, and urine, as well as the total VFA content and VFA profile of rumen fluid and feces, were not affected by ground corn × NH_4Cl interactions (Table 8). None of the ruminal variables were affected by infusion of ground corn or NH_4Cl. Infusion of ground corn decreased ($P < 0.001$) fecal pH, fecal isobutyrate proportion, fecal valerate proportion, and fecal isovalerate proportion, and increased total fecal VFA content and fecal butyrate proportion, relative to no ground corn infusion. Regardless of ground corn inclusion, 2.5 and 5.0 mol of NH_4Cl/d decreased ($P < 0.001$) urine pH compared with no NH_4Cl infusion, with no difference between 2.5 and 5.0 mol of NH_4Cl/d.

DISCUSSION

The aim of this study was to induce hindgut and metabolic acidosis via abomasal infusion of ground corn and NH_4Cl, respectively, and to determine the effects of these physiological states in early-lactating cows. We chose early-lactation cows because these are most prone to metabolic disorders. Importantly, rumen fermentation remained unaffected by the infusion of both ground corn and NH_4Cl, suggesting no appreciable back-flow of infusates into the rumen, particularly of ground corn.

Establishment of Hindgut Acidosis

Fecal total VFA concentration increased upon abomasal infusion of ground corn, suggesting increased hindgut fermentation. Increased hindgut fermentation is also illustrated by the reduction in ATTD of CP, likely the result of increased microbial protein synthesis upon fermentation of a part of the infused ground corn, as well as the increased respiratory quotient values that can arise from increased anaerobic fermentation of dietary carbohydrates (Gerrits et al., 2015). The observed hindgut pH values (i.e., 6.00 and 6.86 pH units for treatment with and without ground corn, respectively) suggest that the increased hindgut fermentation of the infused ground corn resulted in hindgut acidosis, where

Table 6. Blood plasma constituents of early-lactation dairy cows abomasally infused with ground corn and NH_4Cl

	Ground corn (kg/d)		NH_4Cl (mol/d)				*P*-value		
Item	0.0	3.0	0.0	2.5	5.0	SEM	Ground corn	NH_4Cl	Ground corn × NH_4Cl[1]
Urea (mmol/L)	5.5	4.0	4.8	4.6	4.8	0.28	<0.001	0.863	0.548
Insulin (mIU/L)	7.9	6.5	7.2	7.5	6.9	0.77	0.035	0.722	0.447
Triglycerides[2] (mmol/L)	0.090	0.078	0.080	0.084	0.088	0.0062	0.001	0.150	0.005
BHB (mmol/L)	0.65	0.40	0.52	0.54	0.52	0.087	0.002	0.963	0.938
NEFA[3] (mmol/L)	0.33	0.21	0.25	0.25	0.30	0.062	0.006	0.444	0.478
IGF-1 (μg/L)	100	124	114	111	111	33.4	0.005	0.911	0.814
Albumin (g/L)	32	32	32	32	33	0.9	0.718	0.185	0.080
SAA[4] (mg/L)	91	108	108	102	89	23.8	0.239	0.558	0.101
Haptoglobin (g/L)	1.76	1.78	2.01[a]	1.68[b]	1.62[b]	0.182	0.838	0.011	0.445

[a,b]Values with different superscripts indicate a significant difference ($P < 0.05$) between 0.0, 2.5, and 5.0 mol of NH_4Cl/d.

[1]Interaction between 2 levels of ground corn (0.0 or 3.0 kg/d) and 3 levels of NH_4Cl (0.0, 2.5, or 5.0 mol/d). If a significant ground corn × NH_4Cl interaction is found, a different superscript (k–l) in the footnote of that particular variable indicates a significant difference ($P < 0.05$).

[2]Ground corn × NH_4Cl interaction: 0.0 mol of NH_4Cl/d with 0.0 kg of starch/d = 0.086[kl]; 2.5 mol of NH_4Cl/d with 0.0 kg of starch/d = 0.082[k]; 5.0 mol of NH_4Cl/d with 0.0 kg of starch/d = 0.102[l]; 0.0 mol of NH_4Cl/d with 3.0 kg of starch/d = 0.074[k]; 2.5 mol of NH_4Cl/d with 3.0 kg of starch/d = 0.085[k]; 5.0 mol of NH_4Cl/d with 3.0 kg of starch/d = 0.075[k].

[3]Nonesterified fatty acids.

[4]Serum amyloid A.

the pH threshold typically considered for hindgut acidosis ranges from 6.0 to 6.6 (Metzler-Zebeli et al., 2013). Although moderate amounts of starch were fermented in the hindgut, it still caused acidosis because of the lack of salivary buffering and absence of protozoa limiting the ability of the hindgut to self-regulate digesta pH (Gressley et al., 2011).

The lack of simple models to simulate hindgut fermentation has limited the research focus on hindgut acidosis (Plaizier et al., 2018). We were able to induce hindgut acidosis via infusion of ground corn into the abomasum, and the subsequent effects will subsequently be discussed in detail. However, our results might differ from previous findings in which hindgut acidosis was induced via different models. Several studies (e.g., Li et al., 2012; Plaizier et al., 2017) induced hindgut acidosis via a grain-based SARA challenge. This model is effective at increasing the risk of hindgut acidosis, because feeding large amounts of grain, combined with poor rumen mat function associated with SARA conditions, leads to larger amounts of fermentable substrates that bypass rumen fermentation and are subsequently fermented in the hindgut (Plaizier et al., 2018). Our model (i.e., abomasal infusion) induced hindgut acidosis

Table 7. Energy and nitrogen balance (based on TMR + infusion) of early-lactation dairy cows abomasally infused with ground corn and NH_4Cl

	Ground corn (kg/d)		NH_4Cl (mol/d)				*P*-value		
Item	0.0	3.0	0.0	2.5	5.0	SEM	Ground corn	NH_4Cl	Ground corn × NH_4Cl[1]
Respiratory quotient	1.08	1.11	1.11	1.10	1.08	0.017	0.013	0.052	0.214
Metabolic BW[2] ($kg^{0.75}$)	112	111	113[a]	112[a]	110[b]	1.6	0.456	<0.001	0.133
Energy balance (kJ/kg of $BW^{0.75}$ per day, unless stated otherwise)									
GEI[3]	2,195	2,451	2,426	2,360	2,183	118.5	0.013	0.125	0.384
CH_4 production	146	136	153[a]	143[ab]	126[b]	8.5	0.119	0.009	0.127
Energy in manure	682	785	762	747	692	33.9	0.002	0.143	0.545
DEI[4]	1,539	1,645	1,645	1,613	1,518	79.9	0.112	0.275	0.259
MEI[5]	1,366	1,530	1,509	1,472	1,362	80.3	0.016	0.170	0.355
MEI to GEI ratio (%)	62.2	62.3	62.1	62.3	62.4	0.62	0.797	0.876	0.774
Heat production[6]	832	837	860	840	804	20.4	0.802	0.068	0.535
Energy in milk	585	624	604	621	589	24.0	0.040	0.349	0.940
ER total[7]	−51	69	46	15	−35	60.5	0.017	0.378	0.226
ER protein[8]	47	32	60[a]	43[ab]	16[b]	12.2	0.195	0.015	0.190
ER fat[9]	−98	36	−15	−28	−51	51.3	0.001	0.702	0.255
Nitrogen balance (mg/kg of $BW^{0.75}$ per day)									
N intake	3,866	3,876	4,024	3,933	3,656	194.7	0.944	0.102	0.436
N manure	2,619	2,628	2,645	2,645	2,581	120.1	0.883	0.619	0.378
N feces[10]	1,226	1,459	1,445[a]	1,382[ab]	1,200[b]	94.9	0.006	0.042	0.398
N urine[11]	1,394	1,182	1,206[a]	1,262[a]	1,395[b]	60.2	<0.001	0.002	0.303
N milk	854	968	904	932	897	32.1	<0.001	0.462	0.926
N condense + N acid	74	60	69	67	65	12.6	<0.001	0.708	0.655
N balance	319	218	408[a]	290[ab]	106[b]	82.4	0.195	0.015	0.190
N efficiency[12]	22.3	25.3	22.7	23.9	24.9	1.05	<0.001	0.058	0.161

[a,b]Values with different superscripts indicate a significant difference ($P < 0.05$) between 0.0, 2.5, and 5.0 mol of NH_4Cl/d.

[1]Interaction between 2 levels of ground corn (0.0 or 3.0 kg/d) and 3 levels of NH_4Cl (0.0, 2.5, or 5.0 mol/d).

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

[3]Gross energy intake (diet + infusion).

[4]DEI (digestible energy intake) = GEI × apparent total-tract digestibility of GE (% of intake)/100.

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

[6]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).

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

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

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

[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 (%).

but did not affect ruminal conditions, which represents a potentially important difference that could affect the responses observed in the current study.

Effects of Abomasal Ground Corn Infusion and Associated Hindgut Acidosis

Nutrient Intake and Digestibility. Ground corn infusion increased the total intake of several nutrients. This is a direct consequence of the treatment design, where, on average, a realized 2.7 kg/d of ground corn was infused and added to calculated TMR intake. Others (Knowlton et al., 1998; Reynolds et al., 1998) observed a depressed voluntary DMI upon postruminal infusion of ground corn, whereas the DMI of the TMR was not decreased upon ground corn infusion in the current study ($P = 0.161$, results not shown). Likely, the cows in the present study may not have shown a reduction in their voluntary DMI because of the already relatively low DMI of the TMR (i.e., 13.3 kg/d for the treatment without ground corn and NH_4Cl vs. the calculated feed intake capacity of 18.8 kg DM/d at 95% feeding level; Zom et al., 2012). This may have been a result of low palatability of the grass silage, which by design had a low DCAD value (i.e., 184 mEq/kg of DM relative to the Dutch average of 439 mEq/kg of DM; CVB, 2018) and high Cl content (i.e., 19.5 g/kg of DM relative to the Dutch average of 12.3 g/kg of DM; CVB, 2018).

The decreased ATTD of most nutrients, except for fat (not affected) and starch (increased), upon abomasal infusion of ground corn is contrary to previous reports by Knowlton et al. (1998) and Reynolds et al. (2001), and may be the result of multiple factors. The ground corn was infused to bypass the rumen, likely reducing overall fermentation and absorption of the nutrients that were infused. Furthermore, the decreased pH of the hindgut upon ground corn infusion potentially affected the hindgut microbiome. Fibrolytic bacteria are generally inhibited by a lower pH, decreasing fiber fermentation and, subsequently, fiber digestibility. The decreased ATTD of CP was also observed by Gressley and Armentano (2005, 2007) and Gressley et al. (2011), and is likely the result of increased microbial protein synthesis upon fermentation of a part of the infused ground corn. This microbial protein remains undigested and thereby decreases ATTD of N (Ørskov et al., 1969; Owens et al., 1986). The increase in ATTD of starch in response to ground corn infusion may be due to low starch content of the basal diet leading to a lower apparent fecal starch digestibility compared with the digestibility of the infused starch.

Energy Partitioning. The increased GE intake, in combination with decreased ATTD of GE, resulted

Table 8. pH and volatile fatty acids (VFA) of rumen fluid, feces, and urine (pH only) of early-lactation dairy cows abomasally infused with ground corn and NH_4Cl

	Ground corn (kg/d)		NH_4Cl (mol/d)				*P*-value		
Item	0.0	3.0	0.0	2.5	5.0	SEM	Ground corn	NH_4Cl	Ground corn × NH_4Cl[1]
Rumen fluid									
pH	6.31	6.22	6.32	6.26	6.23	0.117	0.119	0.453	0.656
Total VFA (m*M*)	108	108	109	105	110	4.3	0.981	0.297	0.942
VFA (% of total VFA)									
Acetate	71.1	71.0	71.0	71.0	71.0	0.40	0.826	0.997	0.506
Propionate	16.2	16.6	16.2	16.2	16.8	0.35	0.323	0.353	0.815
Butyrate	9.5	9.3	9.6	9.6	9.1	0.24	0.329	0.125	0.104
Isobutyrate	0.63	0.62	0.64	0.62	0.62	0.019	0.883	0.594	0.681
Valerate	1.41	1.42	1.46	1.42	1.36	0.052	0.800	0.459	0.913
Isovalerate	1.14	1.05	1.07	1.11	1.10	0.103	0.242	0.897	0.814
Feces									
pH	6.86	6.00	6.52	6.40	6.37	0.116	<0.001	0.480	0.729
Total VFA (m*M*)	20	28	24	23	24	2.2	0.005	0.964	0.355
VFA (% of total VFA)									
Acetate	78.9	75.0	74.8	77.0	79.1	2.01	0.113	0.374	0.666
Propionate	12.3	11.4	13.1	12.2	10.3	1.19	0.543	0.289	0.586
Butyrate	4.72	11.85	9.16	7.86	7.84	1.09	<0.001	0.564	0.591
Isobutyrate	1.68	0.69	1.16	1.25	1.15	0.103	<0.001	0.581	0.271
Valerate	1.17	0.36	0.83	0.73	0.75	0.054	<0.001	0.397	0.915
Isovalerate	1.18	0.57	0.88	0.87	0.88	0.096	<0.001	0.996	0.730
Urine									
pH	6.74	6.57	7.91[a]	6.10[b]	5.96[b]	0.181	0.170	<0.001	0.531

[a,b]Values with different superscripts indicate a significant difference ($P < 0.05$) between 0.0, 2.5, and 5.0 mol of NH_4Cl/d.

[1]Interaction between 2 levels of ground corn (0.0 or 3.0 kg/d) and 3 levels of NH_4Cl (0.0, 2.5, or 5.0 mol/d).

in increased energy excreted via manure when ground corn was infused. The relative increase in GE intake was more pronounced than the relative decrease in ATTD of GE. In addition, methane energy loss was numerically lower for the infusions containing ground corn. This resulted in a higher MEI with the abomasal infusion of ground corn. The increased MEI could subsequently be used for milk production, which is evident by the observed increase in milk yield and energy secreted in milk, but also for body tissue synthesis (Reynolds et al., 2001), particularly when the increase in MEI arises from glucogenic energy (Nichols et al., 2019). The latter is evidenced by the increased total energy retained as well as energy retained as body fat during ground corn infusion.

Milk yield is largely regulated through the osmotic properties of lactose, synthesis of which requires the transport of glucose from blood into the cytosol of mammary epithelial cells (Cant et al., 2002). There is a considerable capacity for starch digestion in the small intestines via enzymatic hydrolysis resulting in increased glucose supply to the animal (Reynolds, 2006). Glucose concentration in whole-blood increased with ground corn infusion in the current study (Table 5) as well as milk lactose content and lactose yield. This response is in line with the findings of Rius et al. (2010a) and Knowlton et al. (1998). A decrease in milk fat content was observed with abomasal infusion of ground corn in the present study, which is contrary to Knowlton et al. (1998) but in agreement with Rius et al. (2010a). When elevated in circulation, propionate and glucose promote insulin secretion (Bauman and Griinari, 2001). Insulin stimulates uptake of lipogenic precursors and reduces lipolysis in adipose tissue, resulting in reduced circulating fatty acids for milk fat synthesis (Bauman and Griinari, 2001). Although an increase in glucose concentration was observed in response to ground corn infusion in the current study, serum insulin concentrations actually were decreased. Despite the latter, decreased NEFA and BHB concentrations in plasma, decreased acetone and BHB concentrations in milk, and increased energy retention as fat indicate a reduced availability of fatty acids for milk lipid synthesis.

Nitrogen Partitioning. The increased milk protein yield with abomasal infusion of ground corn is contrary to findings of Knowlton et al. (1998), Reynolds et al. (2001), and Rius et al. (2010a). Increased milk protein yield in the present study was mainly caused by increased milk yield, as milk protein content was unaffected. Increased arterial glucose concentrations in response to infusion of ground corn suggest an elevated absorptive glucose supply, which may have supported the increased transfer of dietary N into milk N, in part due to reduced catabolism of AA for gluconeogenesis (Rulquin et al., 2004; Rius et al., 2010a,b).

The clear shift from urinary N to fecal N upon abomasal infusion of ground corn is likely related to fermentation of part of the infused ground corn in the large intestine leading to increased microbial protein synthesis. Because the N source for this microbial protein is partly derived from blood next to N sources in the digesta, this leads to a shift in N excretion from the urine to the feces (Heijnen and Beynen, 1997), as also observed by Reynolds et al. (2001). In cows, the amount of urea excreted in urine is directly proportional, although with considerable variation (Spek et al., 2013), to its concentration in blood (Ciszuk and Gebregziabher, 1994). In support of this, plasma urea decreased in the present study. This can indicate that urea was drawn from plasma to contribute to fecal N at the cost of urine N excretion, because increased hindgut fermentation stimulates assimilation of NH_3 in microbial protein rather than its absorption into the bloodstream, resulting in lower urea production by the liver. Lower plasma urea with ground corn infusion also agrees with reduced AA catabolism in response to increased glucose supply.

Immune Response. We hypothesized that hindgut fermentation of the abomasally infused ground corn would stimulate a systemic inflammatory response. Systemic inflammation can result from the translocation of endotoxins, such as LPS, from the digestive tract to blood. During rapid growth or lysis of bacteria, LPS is released from the bacterial cell walls and animal tissue becomes exposed to these toxic components (Hurley, 1995; Wells and Russell, 1996). Several studies have observed that grain-induced SARA challenges increase the free LPS content of digesta in the large intestine and feces (e.g., Li et al., 2016; Qumar et al., 2017), likely as a result of hindgut acidosis caused by increased amounts of starch that bypasses rumen fermentation and digestion in the small intestine (Gressley et al., 2011).

The simpler structure makes the hindgut epithelium more susceptible than the rumen epithelium to barrier damage and toxin translocation under acidic conditions (Tao et al., 2014), impacting on the animal. The barrier function of the monolayer epithelium of the large intestine for LPS may thus be compromised relatively easily (Emmanuel et al., 2007; Plaizier et al., 2012), and high LPS in the large intestine may therefore pose a risk for systemic inflammation (Plaizier et al., 2018). However, the absence of effect of abomasally infused ground corn on the plasma concentrations of the acute-phase proteins haptoglobin, albumin, and SAA in the current study suggests that hindgut fermentation of the

infused ground corn did not result in a systemic inflammatory response. Although systemic inflammation was not detected by these plasma markers, this does not preclude the possibility that the epithelium of the large intestine became (or would become, in the longer term) more susceptible to damage or increased permeability (not tested in the current study).

Establishment of Metabolic Acidosis

Cows receiving 5.0 mol of NH_4Cl without ground corn or 2.5 and 5.0 mol of NH_4Cl/d with ground corn had a blood pH below 7.40, a blood HCO_3 concentration below 25.0 mmol/L, and a negative base excess. This blood profile suggests that the cows experienced subacute metabolic acidosis (Schotman, 1971). The absorption of Cl from the gastrointestinal tract reduces the strong ion difference in body fluids. In an effort to maintain the acid-base equilibrium, HCO_3 is depleted and H^+ is released, resulting in decreased blood pH (Hu and Murphy, 2004). The observed interactions between ground corn and NH_4Cl for blood pH, HCO_3, partial pressure of CO_2 (pCO_2; tendency only), base excess, and total CO_2, indicate that the effect of NH_4Cl infusion on the acid-base status was affected by the presence of abomasal ground corn infusion. This is likely related to increased VFA absorption from the hindgut during ground corn 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 in beef steers (Brown et al., 2000) and decreased blood pH. This mechanism is likely valid for the hindgut as well, especially when excessive fermentation takes place. Abomasal ground corn infusion induced hindgut fermentation in the present study, which explains the stronger effect of NH_4Cl infusion on the acid-base status of dairy cows in the presence of ground corn.

Irrespective of treatment, we observed positive relationships (n = 36) between the overall DCAD value (in mEq/kg of DM; basal diet + infusion treatments; Table 1) and blood pH ($r = 0.72$, $P < 0.001$), blood HCO_3 concentration ($r = 0.80$, $P < 0.001$), and blood pCO_2 ($r = 0.60$, $P < 0.001$). This is in agreement with the relationships observed by Hu and Murphy (2004). Blood HCO_3 pattern follows blood pH response according to the Henderson-Hasselbalch equation: blood pH = $6.1 + \log_{10} [HCO_3/(0.03 \times pCO_2)]$ (Hu and Murphy, 2004). In clinically healthy cows, acid-base disturbances are compensated by various regulatory mechanisms (Gärtner et al., 2019), with the kidneys fulfilling a prominent role through ion transport mechanisms and through reabsorption and synthesis of HCO_3 and the accompanying excretion of H^+ (Enemark et al., 2002). The efficient renal elimination of excess of anions, such as Cl upon infusion of NH_4Cl, causes a reduction in urinary pH (Wang et al., 2018), which we did observe in response to NH_4Cl infusion in the current study.

Effects of Abomasal NH_4Cl Infusion and Associated Metabolic Acidosis

Nutrient Intake and Digestibility. The tendency toward decreased DMI of the TMR with infusion of 5.0 mol of NH_4Cl/d, irrespective of ground corn infusion, appears mainly due to the successful induction of metabolic acidosis. Zimpel et al. (2018) recently concluded that the decrease in DMI of dry pregnant cows in response to diets with negative DCAD was mediated by metabolic acidosis and not by the presence of acidogenic products or salts containing Cl in dietary DM (i.e., palatability). This conclusion was supported by a linear relationship between blood pH and DMI. Similarly, in the present study, blood pH was positively related to both total DMI ($r = 0.64$, $P = 0.003$) and DMI of the TMR ($r = 0.49$, $P < 0.001$).

Iwaniuk and Erdman (2015) reported a linear positive relation between dietary DCAD concentration (ranging between 13 and 436 mEq/kg of DM) and the ATTD of DM and NDF, where changes in fiber digestibility due to variation in DCAD were likely the result of changes in ruminal pH (e.g., Erdman et al., 1982; West et al., 1987). In contrast, we did not observe an effect of NH_4Cl on the ATTD of DM and NDF, and ruminal pH, likely due to the method of infusion of NH_4Cl into the abomasum. Increased digestibility of CP, crude fat, and starch at the highest level of NH_4Cl infusion compared with no NH_4Cl infusion was likely the result of the lower TMR DMI, allowing a longer retention time in the rumen and increased ruminal fermentation of these components (Colucci et al., 1989).

Energy Partitioning. The lower metabolic BW of the cows when they received 5.0 mol of NH_4Cl/d compared with 2.5 mol of NH_4Cl/d is likely a result of the lower DMI and subsequently less feed in the gastrointestinal tract and lower digesta content weight. Milk yield as well as energy secreted in milk were not affected by NH_4Cl, which is in line with Chan et al. (2005), Roche et al. (2003, 2005), and Apper-Bossard et al. (2006), although others have reported linear increases in milk yield with increasing DCAD concentration in dietary DM (Tucker et al., 1988; Apper-Bossard et al., 2010). The meta-analyses of Hu and Murphy (2004) and more

recently of Iwaniuk and Erdman (2015) demonstrated a nonlinear milk yield response to increasing DCAD concentration in dietary DM.

The lack of effect of NH_4Cl on milk fat content and yield in the present study is in agreement with Tucker et al. (1988), Chan et al. (2005), and Roche et al. (2003), but contrary to Iwaniuk and Erdman (2015), Apper-Bossard et al. (2006, 2010) and Roche et al. (2005), who all reported increases in milk fat content and yield with increasing DCAD values. These previous studies manipulated the DCAD value via the addition of cation or anion sources to the diet fed, potentially affecting rumen fermentation. Iwaniuk and Erdman (2015) reported a positive linear relation between the DCAD value and ruminal pH and the molar proportions of acetate and butyrate, all of which have been associated with increased milk fat content (Kolver and de Veth, 2002; Jenkins et al., 2014). In the current study, however, the DCAD value of the basal diet was similar for all treatments, and NH_4Cl was infused into the abomasum, preventing effects at the rumen level and its consequences downstream on milk fat synthesis. The decrease in milk lactose content with increasing levels of NH_4Cl infusion is contrary to Roche et al. (2003) and Apper-Bossard et al. (2006) but in agreement with Wang et al. (2018). The main constituents involved in maintaining the osmotic pressure of milk, which is always closely related to the osmotic pressure of blood, are lactose, Na, K, and Cl (Bijl et al., 2013). Wang et al. (2018) observed a linear increase in serum Cl concentrations with increasing dose of NH_4Cl in ruminal infusions, and Hu and Murphy (2004) reported a positive linear relation between dietary Cl content and Cl concentration in the blood. Concentration and electrical gradients in the mammary gland between extra- and intracellular fluid and milk tend to drive Cl into milk (Peaker, 1983; Shennan and Peaker, 2000). Although not measured in the current study, the abomasal infusion of NH_4Cl likely resulted in increased Cl concentrations in both blood and milk. Bijl et al. (2013) reported an inverse relationship between milk lactose content and milk Cl concentrations ($r = -0.66$, $P < 0.01$), potentially explaining the decreasing milk lactose content with increased levels of NH_4Cl infusion observed in the present study.

Nitrogen Partitioning. The unaffected milk protein content and yield with infusion of NH_4Cl is in agreement with the findings of Tucker et al. (1988), Chan et al. (2005), and Iwaniuk and Erdman (2015), and is likely due to the unaffected N intake. Our results indicate that metabolic acidosis induced by high levels of abomasal infusion of NH_4Cl stimulates partitioning of body N toward urinary N excretion, because N balance decreased with infusion of NH_4Cl and fecal N shifted to urinary N excretion.

Immune Response. Plasma haptoglobin, but not plasma albumin or SAA, decreased in response to infusion of NH_4Cl. Haptoglobin is an acute-phase protein that plays an essential role in immune cell function and tissue repair (Roche et al., 2013). Haptoglobin production is initiated by proinflammatory cytokines released at sites of infection or tissue damage (Carroll et al., 2009). The concentrations of haptoglobin in the present study were well above concentrations of 0.8 to 1.0 g/L, regardless of treatment, suggesting that the early-lactation cows in the present study would have to be classified as animals at high risk for periparturient diseases (Huzzey et al., 2009; Dubuc et al., 2010). Haptoglobin was not associated with SCC or $\log_{10}$ SCC (both $P > 0.100$), suggesting that mastitis was not the basis for these relatively high haptoglobin concentrations, in agreement with the lack of clinical signs or treatment of mastitis (or any other disease) during the experiment. It is possible that the metabolic challenge induced by infusion of high doses of NH_4Cl may have affected immune responses such that haptoglobin production was inhibited. However, the mode of action behind this hypothesis cannot be elucidated based on the measurements in the current study.

CONCLUSIONS

Abomasal infusion of NH_4Cl affected the acid-base status of dairy cows, and this occurred more strongly when ground corn was also infused in the abomasum. Metabolic acidosis was observed with abomasal infusion of 5.0 mol of NH_4Cl/d in the absence of ground corn and with abomasal infusion of both 2.5 and 5.0 mol of NH_4Cl/d in the presence of ground corn. Metabolic acidosis increased urinary N excretion while decreasing fecal N excretion, and decreased energy retained as protein. Abomasal ground corn infusion resulted in increased hindgut fermentation (indicated by increased fecal VFA concentrations) and induced hindgut acidosis. Hindgut acidosis was associated with decreased ATTD of most nutrients. A systemic inflammatory response was not observed, suggesting that the hindgut epithelium was not severely affected by acidic or barrier damage. Abomasal infusion of ground corn increased milk yield, milk protein and lactose yield, N use efficiency, and energy retained in total as well as in fat, but reduced milk fat content. Overall, in this short-term experiment, induced hindgut acidosis was not associated with negative effects on production performance and energy balance, whereas induced metabolic acidosis tended to decrease feed intake and

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body protein retention; however, long-term studies are needed to further elucidate the impact of hindgut and metabolic acidosis in dairy cattle.

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