Effects of metritis treatment strategies on health, behavior, reproductive, and productive responses of Holstein cows

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ABSTRACT

Our objectives were to compare the effects of ceftiofur crystalline free acid (CCFA) and ampicillin trihydrate (AMP) treatments of cows diagnosed with metritis on uterine health, behavior, reproductive, and productive responses. A controlled randomized clinical trial was designed. Metritis was defined as vaginal discharge (VD) = 5 (fetid, watery, red/brown) within 21 d in milk (DIM) and rectal temperature (RT) <39.5°C, whereas VD = 5 and RT ≥39.5°C was defined as puerperal metritis. On the day of diagnosis (d 0), cows were paired by parity and severity of metritis (metritis vs. puerperal metritis) and assigned randomly to the AMP and CCFA treatments. Cows enrolled in the AMP (n = 308) treatment were moved to a nonsalable-milk pen, where they were treated once daily for 5 d, and were moved back to their original pen 72 h after the last treatment (d 7). Cows enrolled in the CCFA (n = 310) treatment remained in their original pen and received 2 treatments of CCFA, 72 h apart. Rectal temperature was measured daily from d 0 to 6 and on d 11. Vaginal discharge was evaluated on d 4, 6, and 11 to assess cure. Cure was defined as the absence of treatment with additional antimicrobial before experiment d 11, VD <5, and RT <39.5°C. Cows were examined at 28 ± 3 DIM for purulent VD (PVD) and at 35 ± 3 DIM for cytological endometritis. Pregnancy was diagnosed at 40 ± 3 and 60 ± 7 d after first and second artificial inseminations. Cure of metritis did not differ between treatments on d 11 (AMP = 64.6 ± 3.1, CCFA = 63.5 ± 3.1%). Cows treated with AMP had greater RT from experiment d 1 to 6 compared with cows treated with CCFA (AMP = 39.1 ± 0.02, CCFA = 39.0 ± 0.02°C). Cows in the AMP treatment had greater prevalence of PVD at 28 ± 3 DIM (AMP = 82.6 ± 2.3, CCFA = 74.4 ± 2.7%) and tended to have greater prevalence of cytological endometritis at 35 ± 3 DIM (AMP = 77.8 ± 6.2 vs. CCFA = 61.7 ± 7.5%) than CCFA-treated cows. Treatment did not affect the hazard of pregnancy among multiparous cows; however, among primiparous cows, CCFA treatment reduced the hazard of pregnancy and increased the median days to pregnancy (AMP = 145 vs. CCFA = 169 d). Finally, average daily milk yield up to 14 wk postpartum was not affected by treatment (AMP = 38.0 ± 0.4, CCFA = 37.5 ± 0.4 kg). We conclude from the current experiment that CCFA was more effective in reducing RT and improving uterine health of metritic cows; however, the improved hazard of pregnancy of primiparous cows treated with AMP is important and warrants further investigation.

Key words: dairy cattle, metritis, ampicillin, ceftiofur

INTRODUCTION

Metritis is a uterine infectious disease resulting in fetid, watery, reddish-brownish vaginal discharge (VD; Chenault et al., 2004), whereas puerperal metritis is characterized by the presence of such VD accompanied by systemic signs such as pyrexia (≥39.5°C), dullness, anorexia, and dehydration (Sheldon et al., 2006). In the USA, the incidence of metritis ranges from 20 to 40% (Haimerl and Heuwieser, 2014). Metritis is commonly associated with impaired reproduction (Ribeiro et al., 2016), greater culling rates (Bartlett et al., 1986), visceral pain (Stojkov et al., 2015), and economic losses of approximately $358 per case (Overton and Fetrow, 2008). Due to the diverse etiology of metritis, broad-spectrum antimicrobials are warranted as the main therapeutic strategy for this disease (Haimerl et al., 2017). Presently, the most commonly used antimicrobials are systemic penicillin, oxytetracycline, ampicillin, and ceftiofur (Haimerl and Heuwieser, 2014). The use of ceftiofur, however, has been associated with
increased resistance to ceftriaxone, which is a third-generation cephalosporin strictly available for human use (Tragesser et al., 2006). Thus, some have proposed the use of other classes of drugs, such as penicillin, as alternatives to reduce the use of ceftiofur in lactating dairy cows.

Ampicillin (AMP) is an aminopenicillin that can be used as an alternative treatment for metritis in dairy cattle (Lima et al., 2014). The penicillins and cephalosporins (e.g., ceftiofur) are referred to as β-lactam antimicrobials because they present a basic β-lactam ring structure (Shaw, 2015). All β-lactam antimicrobials interfere in the transpeptidation reactions that occur during the formation of the bacterial cell wall, leading to bacterial death (Beauduy and Winston, 2015). In contrast to ceftiofur, a third-generation cephalosporin more stable against bacterial β-lactamases, the β-lactam ring of ampicillin can be hydrolyzed by bacterial β-lactamases, inactivating its antimicrobial properties (Beauduy and Winston, 2015). In the USA, ceftiofur hydrochloride, ceftiofur crystalline free acid (CCFA), and injectable oxytetracycline are the only antimicrobials labeled for metritis treatment (Espada-mala et al., 2018).

Ceftiofur is usually the preferred option for treatment of metritis because it is labeled for this purpose and has proven clinical efficacy against this disease (McLaugh-lin et al., 2012). In addition, ceftiofur does not require milk withdrawal (Jeon et al., 2018), abolishing the need for group changes, whereas AMP requires a 48-h milk withdrawal and movement to a nonsalable-milk pen. Schirmann et al. (2011) demonstrated that prepartum cows that were regrouped had decreased DMI and were more likely to be displaced at the feed bunk by other cows. Thus, we hypothesized that another advantage of the treatment of cows with metritis with CCFA compared with AMP is the stable hierarchical order to which they are exposed because they are not moved to a nonsalable-milk pen.

We hypothesized that CCFA is an effective treatment strategy for metritis that results in greater likelihood of cure, improved uterine health, and improved reproductive and productive performances compared with cows treated with AMP. Our objectives were to determine the effects of treating cows diagnosed with metritis with CCFA on cure, uterine health, concentration of metabolites and behavior during treatment, reproductive and productive performances, compared with AMP treatment. A secondary objective of this experiment was to determine the differences in the outcomes cited previously between cows diagnosed with metritis and cows that were not diagnosed with clinical diseases in the first 21 DIM.

**MATERIALS AND METHODS**

All procedures involving animals were approved by the Animal Care and Use Committee of the University of Florida, Gainesville (protocol no. 201710099).

**Animals, Housing, and Management**

This experiment was conducted from December 2017 to July 2018 in 2 commercial dairy herds in south Florida. Farm A had approximately 2,300 lactating cows, and farm B had approximately 2,500 lactating cows. During the experiment, the rolling herd average milk yield were 10,333 kg/cow in farm A and 12,049 kg/cow in farm B.

In farm A, all parous cows were housed in freestall barns starting at approximately 3 wk prepartum. The prepartum nulliparous animals were housed in open-lot corrals from 60 to 30 d before the expected calving date and were subsequently moved to freestall barns according to the availability of space in the prepartum pen. In farm B, nulliparous and parous animals were housed in freestall barns during the last 4 wk prepartum. In farms A and B, primiparous and multiparous cows were moved to freestall barns after calving, where they were housed together. The freestall barns were sand-bedded and were equipped with fans over the stalls and feeding alley and sprinklers over the feeding alley.

In farms A and B, cows were fed a TMR diet that met or exceeded the requirements of 650-kg Holstein cows producing 40 kg/d of 3.5% fat-corrected milk. During the first 21 d postpartum, the main ingredients of the diets in farms A and B were corn silage, haylage, corn and soybean meals, fat supplement, enzyme blend, minerals, and molasses. The diets of the nonsalable-milk pens in farms A and B were the same as the diets offered to postpartum cows, except that in farm A the diet fed in the nonsalable-milk pen did not contain molasses. Two TMR samples were collected monthly from the postpartum (farm A and B) and nonsalable-milk (farm A) pens. Nutrient contents of the rations are depicted in Table 1.

Cows were milked twice daily at farm A (0600 and 2200 h) and thrice daily at farm B (0700, 1500, and 2300 h), whereas cows in the nonsalable-milk pen were milked twice daily (farm A = 0530 and 1600 h; farm B = 0500 and 1630 h). Information regarding calving difficulty (1 = unassisted parturition; 2 = minimal assistance; 3 = moderate assistance; 4 = severe assistance; 5 = cesarean section or fetotomy), calf sex, twins, stillbirth (calves that were dead at delivery or that died within 24 h of delivery), retained fetal membranes (failure to detach the fetal membranes
within 24 h after calving), and mastitis were recorded by farm personnel on the herds’ management software (7.15; Dairy Records Management System, Chapel Hill, NC) and retrieved by study personnel. Cows delivering twins and stillborn calves, and cows that had dystocia (calving difficulty ≥1), were classified as having calving disorders. Retained fetal membranes were diagnosed by study personnel based on visual inspection and, when necessary, vaginal palpation 24 h after calving. Mastitis was diagnosed by farm personnel and defined as abnormal milk (serous milk or presence cloths, blood, or pus) or positive California Mastitis Test (scores 1, 2, and 3; Kandeel et al., 2018).

**Experimental Design, Treatments, and Cure Definition**

Cows in both farms were examined with the Metricheck device (Simcro, Hamilton, New Zealand) by study personnel at 4, 6, 8, 10, and 12 DIM. In addition, farm personnel determined the rectal temperature (RT) of cows daily from calving to 10 DIM and study personnel examined cows with pyrexia (RT ≥ 39.5°C) on the same day using the Metricheck. Cows with retained fetal membranes were examined daily by study personnel using the Metricheck until they had VD = 5 or until they released fetal membranes. Cows with VD = 4 at 12 DIM continued to be examined daily until 21 DIM by study personnel using the Metricheck. Seven cows (farm A = 4, farm B = 3) enrolled in the AMP treatment were enrolled between 13 and 17 DIM, and 12 cows (farm A = 8, farm B = 4) enrolled in the CCFA treatment were enrolled between 13 and 17 DIM.

The Metricheck device was disinfected with a chlorhexidine solution (Bimeda Inc., Oakbrook Terrace, IL) between cows. The vulva was cleaned thoroughly with paper towel and alcohol (70% vol/vol) before insertion of the Metricheck to retrieve the VD. The VD retrieved was classified as follows (adapted from Chenault et al., 2004): 1 = normal lochia; 2 = cloudy mucous; 3 = mucopurulent with less than 50% pus content; 4 = mucopurulent with more than 50% pus content; and 5 = fetid, watery, reddish or brownish discharge. Cows presenting VD = 5 were classified as having metritis. Upon diagnosis of metritis, RT was measured using a digital thermometer (GLA M700 Digital Thermometer, San Luis Obispo, CA) that was calibrated daily. Cows with VD = 5 and RT ≥39.5°C were diagnosed with puerperal metritis.

Before the Metricheck examination at 4 DIM, vulvovaginal laceration score was recorded for each cow. Vulvovaginal laceration scores were defined as follows (Vieira-Neto et al., 2016): 0 = absence of laceration, 1 = presence of laceration <2 cm at the dorsal commissure or lateral walls of the vulva, and 2 = presence of laceration ≥2 cm at the dorsal commissure of the vulva or lateral walls of vulva, vagina, or both.

Upon diagnosis of metritis (experiment d 0), within farm, cows were assigned randomly according to parity (primiparous = 252, multiparous = 366) and severity of the disease (metritis = 421, puerperal metritis = 197) to receive one of the 2 antimicrobial treatments. Randomization was performed with the GraphPad program (8.0.0, GraphPad Software Inc., La Jolla, CA) through an online tool (0.6.0, QuickCalcs, GraphPad Software Inc.). Cows assigned to the AMP (n = 308) treatment were moved to a nonsalable-milk pen, where they were treated with ampicillin trihydrate (11 mg/kg of BW i.m.; Polyflex, Boehringer Ingelheim Vetmedica, St. Joseph, MO) once daily for 5 consecutive days. On d 7 after enrollment, cows enrolled in the AMP treatment were moved back to the postpartum pen.

<table>
<thead>
<tr>
<th>Nutrient content, mean ± SD¹</th>
<th>Farm A</th>
<th>Farm B</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP, % of DM</td>
<td>17.4 ± 1.67</td>
<td>16.8 ± 0.91</td>
</tr>
<tr>
<td>NDF, % of DM</td>
<td>27.7 ± 1.78</td>
<td>25.8 ± 2.35</td>
</tr>
<tr>
<td>Fatty acids, % of DM</td>
<td>4.9 ± 0.47</td>
<td>5.8 ± 0.40</td>
</tr>
<tr>
<td>NFC, % of DM</td>
<td>43 ± 1.70</td>
<td>45.2 ± 2.10</td>
</tr>
<tr>
<td>TDN, at 1× % of DM</td>
<td>70.2 ± 1.43</td>
<td>72.3 ± 2.05</td>
</tr>
<tr>
<td>NE₇₆, Mocal/kg</td>
<td>1.43 ± 0.03</td>
<td>1.48 ± 0.04</td>
</tr>
<tr>
<td>NE₉₀, Mocal/kg</td>
<td>0.97 ± 0.04</td>
<td>1.03 ± 0.05</td>
</tr>
<tr>
<td>NE₉₅, Mocal/kg</td>
<td>1.52 ± 0.04</td>
<td>1.59 ± 0.06</td>
</tr>
<tr>
<td>Particles on 19-mm sieve, % of TMR</td>
<td>20.1 ± 0.09</td>
<td>19.2 ± 0.08</td>
</tr>
<tr>
<td>Particles on 8-mm sieve, % of TMR</td>
<td>34.8 ± 0.09</td>
<td>33.9 ± 0.08</td>
</tr>
<tr>
<td>Particles on 1.9-mm sieve, % of TMR</td>
<td>16.9 ± 0.02</td>
<td>17.4 ± 0.01</td>
</tr>
<tr>
<td>Particles on bottom sieve, % of TMR</td>
<td>28.3 ± 0.03</td>
<td>29.5 ± 0.05</td>
</tr>
</tbody>
</table>

¹Two TMR samples were collected monthly from the postpartum (farms A and B) and nonsalable-milk (farm A) pens. The samples were sent for chemical analyses at the end of the experiment.

pen. Cows assigned to receive CCFA ($n = 310$) were treated with ceftiofur crystalline free acid (6.6 mg/kg of BW s.c.; Excede Sterile Suspension, Zoetis, Madison, NJ) twice, 72 h apart. Upon diagnosis of metritis, the approximate BW of each cow was determined using a heart-girth tape (Weight-By-Breed Dairy Management Tape, Nasco, Fort Atkinson, WI) for calculation of the volume of the antimicrobial to be administered.

Healthy ($HTY; n = 587$) cows were paired with cows diagnosed with metritis according to their parity and calving date. Healthy cows were not diagnosed with any clinical health disorder within 21 DIM and had VD $\leq 4$ in all Metricheck examinations. To compare the concentrations of metabolites and behavior between cows with metritis and HTY cows, we assigned HTY cows experiment d 0 as the average DIM at metritis diagnosis of their counterparts (cows enrolled in the AMP and CCFA treatments) within farm, week of calving, and parity.

Vaginal discharge of cows treated with AMP and CCFA was evaluated on d 4, 6, and 11 by study personnel using the Metricheck device. Additionally, RT was measured daily from d 0 to 6 and on d 11. Rectal temperature was measured immediately after the cows returned from the a.m. milking, when they were restrained in self-locking headlocks. Among the 421 cows diagnosed with metritis, 151 (35.9%) and 166 (39.4%) cows had at least 1 case of fever by d 4 and d 6, respectively. Therefore, cure of metritis was defined as (1) absence of additional antimicrobial treatment (escape therapy) by d 11 and VD $< 5$, and (2) absence of escape therapy by d 11, VD $< 5$, and RT $< 39.5°C$. Farm personnel were allowed to treat cows enrolled in the experiment when they considered that their clinical condition did not improve or was life-threatening.

Automated Monitoring Device and Behavior Responses

Automated monitoring devices (HR-LDn tags, SCR Engineers Ltd., Netanya, Israel) were fitted on a subset of cows ($n = 719$). 21 d before the expected calving date and were removed at 80 DIM. In both farms, every cow that was moved to the prepartum pen from April to June 2018 received an automated monitoring device. The automated monitoring device, fitted on the cranial third of the neck, determines cow-state (side lying, resting, high and medium activity, rumination, eating, grazing, walking, and panting) every minute and was validated by our group (Merenda et al., 2020). Among cows fitted with automated monitoring devices, 122 were diagnosed with metritis (AMP: primiparous = 21, multiparous = 42; CCFA: primiparous = 16, multiparous = 43), and 68 were diagnosed with puerperal metritis (AMP: primiparous = 19, multiparous = 19; CCFA: primiparous = 18, multiparous = 12). Of the cows fitted with automated monitoring devices, 374 were classified as HTY (primiparous = 72, multiparous = 302). Among cows fitted with automated monitoring devices, 42 were not used because they did not undergo Metricheck exams 5 times, 6 were culled before calving, and 71 were not diagnosed with metritis or puerperal metritis but presented other clinical diseases (e.g., mastitis, digestive problems, postpartum complications) or left the herd before 21 DIM, and 36 did not calve during the experiment. The data from 564 cows (farm A = 266, farm B = 298) were used for assessment of rumination, activity, feeding, and resting time.

Blood Sampling, Metabolites, and Body Condition Score

Blood was sampled on d 0, 1, 2, 6, 7, 8, and 14 from a randomly selected subset of cows (AMP = 95, CCFA = 106, HTY = 122) by puncture of the coccygeal vein or artery using evacuated tubes containing K2 EDTA (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). This sampling schedule was adopted because on d 0 and 7 cows treated with AMP were regrouped, whereas CCFA-treated cows were not regrouped. The samples were maintained in a cooler with ice from collection until centrifugation (2,000 $\times$ g for 15 min at 4°C) for separation of plasma. Plasma aliquots were stored at $-80°C$ until the assays were performed.

Concentrations of glucose, nonesterified fatty acids (NEFA), and BHB were analyzed using an RX Dayton Bench-Top Clinical Biochemical Analyzer (kits no. GL3981, FA115, and RB1007; Randox Laboratories Ltd., Crumlin, UK). The interassay coefficients of variation (CV) were 1.4% for glucose, 3.4% for NEFA, and 2.4% for BHB. All assays were organized so that each assay included all the samples from the same cow (d 0, 1, 2, 6, 7, 8, and 14) and equal number of cows from each treatment (AMP, CCFA, and HTY). Concentrations of haptoglobin were determined using a colorimetric assay as described previously by Cooke and Arthington (2013), and the intra- and interassay CV were 7.9 and 11.1%, respectively.

All cows were scored for body condition at enrollment and at 35 ± 3 and 63 ± 3 DIM. In addition, cows that were fitted automated monitoring devices were scored for body condition at 21 d before the expected calving date and at calving. Body condition was scored from 1 = emaciated to 5 = obese, in 0.25-unit increments (Ferguson et al., 1994).
Temperature–Humidity Index

Weatherproof data loggers (HOBO U23 Pro v. 2 External Temperature Data Logger U23-004, Onset Computer Corp., Bourne, MA) were used to determine temperature and relative humidity during the experiment. Data loggers were placed in each of the postpartum and nonsalable-milk pens in which cows enrolled in the experiment were housed. Daily temperature–humidity index (THI) was determined using the following formula:

$$\text{THI} = (0.8 \times \text{daily temperature in °C}) + [\text{RH} \times (\text{daily temperature in °C} - 14.4)] + 46.4.$$  

Relative humidity (RH) was determined using the following formula (Abbott and Tabony, 1985):

$$\text{RH} = \frac{\exp\left(\frac{1.8096 \times (17.2694 + \text{T}_{\text{dew}})}{237.3 + \text{T}_{\text{dew}}}\right) - 1}{\exp\left(\frac{1.8096 \times 237.3}{237.3 + \text{T}_{\text{dew}}}\right) - 1} \times 100,$$

where $T$ = dry bulb temperature (in °C) and $T_{\text{dew}}$ = dew point temperature (in °C). We then calculated the percentage of days that each cow was exposed to $\text{THI} \geq 68$, within 7 and within 28 d after enrollment.

Purulent Vaginal Discharge and Cytological Endometritis

Cows were examined at 28 ± 3 DIM with the Metrichheck device for purulent vaginal discharge (PVD). Cows were classified as having PVD when $V_D \geq 4$ (0 = absence of discharge, 1 = clear mucus, 2 = mucus with flecks of pus, 3 = mucopurulent discharge, 4 = purulent discharge, and 5 = foul-smelling discharge; Denis-Robichaud and Dubuc, 2015).

Endometrial cytology samples were collected from a subset of cows (AMP = 50, CCFA = 51, HTY = 50) at 35 ± 3 DIM. Cows were selected randomly according to farm, parity, and day of enrollment. Uterine samples were collected using a stainless-steel instrument coupled with a cytobrush (Medscand Cytobrush Plus Endocervical Sampler, CooperSurgical Inc., Trumbull, CT; Kasimanickam et al., 2004). After sample collection, the cytobrush was rolled on a microscope glass slide (Gold Seal Plain Microscope Slide, Thermo Fisher Scientific, Waltham, MA) and immediately air-dried. The slides were stained (Stain Kit JorVet Dip Quick, Thermo Fisher Scientific) within 5 h of sample collection. One researcher, blind to treatments, read all the slides. Two hundred cells were counted, and the percentage of polymorphonuclear leukocytes (PMNL) relative to total endometrial cells was recorded. Cows with $\geq 6\%$ of cells classified as PMNL were considered to have cytological endometritis (Denis-Robichaud and Dubuc, 2015).

Resumption of Cyclicity

Ovaries from a subset of cows (AMP = 99, CCFA = 107, HTY = 105) were examined by transrectal ultrasonography (5 MHz; Easi-Scan, BCF Systems, Livingston, UK) at 35 ± 3 DIM and 49 ± 3 DIM in farm A and 39 ± 3 and 53 ± 3 DIM in farm B. Cows with at least 1 corpus luteum >15 mm in diameter at least 1 of the 2 exams were considered to have resumed cyclicity. Cows were selected randomly for transrectal ultrasonography according to farm, parity, and day of enrollment. Resumption of cyclicity of cows fitted with an automated monitoring device (HR-LDn tags, SCR Engineers Ltd.; AMP = 101, CCFA = 89, HTY = 374) was determined based on estrus events recorded by the DataFlow2 system (SCR Engineers Ltd.) by 35 ± 3 DIM and 49 ± 3 DIM in farm A and by 39 ± 3 and 53 ± 3 DIM in farm B.

Reproductive Management, Reproductive Performance, and Milk Yield

In both farms, cows were enrolled in a presynchronization protocol (2 injections of PGF$_{2\alpha}$, 14 d apart) at 35 ± 3 and 39 ± 3 DIM in farms A and B, respectively. At 14 (farm A) and 10 (farm B) d after the second PGF$_{2\alpha}$ injection of the presynchronization protocol, cows were enrolled in the Cosynch72 protocol (GnRH, 7 d later PGF$_{2\alpha}$, 72 h later GnRH and fixed-time AI) at 63 ± 3 DIM in both farms. After the first postpartum AI, cows were reinseminated when detected in estrus by farm personnel. On farm A, cows diagnosed not pregnant were enrolled in the Cosynch72 protocol, whereas on farm B cows diagnosed not pregnant were treated with PGF$_{2\alpha}$ and, if not detected in estrus, enrolled in the Ovsynch protocol 7 d later. Visual detection of estrus was performed daily by farm personnel with the aid of tail chalk.

Pregnancy was diagnosed at 40 ± 3 d after AI by palpation per rectum of the uterine contents. At 60 ± 3 d after the first and second postpartum AI, pregnant cows were examined by study personnel via transrectal ultrasound (Easi-Scan, BCF Systems, Livingston, UK) for identification of an embryo with heartbeat. After the second pregnancy diagnosis, pregnancy reconfirmation was conducted by farm personnel at approximately 200 d of gestation. Data regarding AI technician and
reproductive outcomes were obtained from the on-farm software. All the cows enrolled in the experiment were followed until they completed 305 DIM or left the herd.

Individual milk yield of cows in herds A and B was recorded monthly through individual parlor milk meters. Cows that were in the nonsalable-milk pen on the day of milk measurement also had their individual milk production recorded. Weekly predicted milk weights from calving to 14 wk postpartum were generated by the on-farm herd management software (19.6; Dairy Records Management System) based on parity, previous milk production, stage of lactation, and projected lactation curve. Individual weekly milk yield was recorded from calving until 14 wk postpartum or until the cow left the herd.

Statistical Analyses

This was a controlled randomized clinical trial. Sample size calculation for metritis cure was performed using MedCalc (MedCalc Software Ltd., Ostend, Belgium). According to previously published literature (McLaughlin et al., 2012; Lima et al., 2014), the expected cure on d 11 to 14 after diagnosis would be between 75 and 85%. We determined that a sample size of 304 cows per treatment was necessary to detect a 7-percentage-unit difference in cure on d 11 after diagnosis (e.g., CCFA = 75 vs. AMP = 68%) while preventing type I (α = 0.05) and type II (β = 0.20) errors. In addition, we determined that 40 cows per treatment were necessary to detect a 25 min/d difference in feeding time between the AMP and CCFA treatments when the feeding time of CCFA cows is 135 min/d and the SD is 55 min/d. Huzzey et al. (2007) had demonstrated that the difference in daily feeding time between healthy cows and cows diagnosed with mild metritis was between 22 and 25 min/d on wk −1 and 1 relative to calving. Furthermore, through a post hoc analysis, we determined that the sample size necessary to detect (α = 0.05, β = 0.80) a 7.5-percentage-unit difference in pregnancy per AI to first postpartum service between AMP and CCFA was 275 cows per treatment, when the pregnancy per AI of AMP-treated cows is 25%.

Using post hoc sample size calculations, we determined the statistical differences that could be detected taking into consideration the interactions between treatment and parity and between treatment and severity of the disease. When the interaction between treatment and parity was used, the smallest sample size was 125 primiparous cows in the AMP treatment and was sufficient to detect a 10.75-percentage-unit difference in cure on d 11 after diagnosis (e.g., CCFA = 78.75 vs. AMP = 68%) while preventing type I (α = 0.05) and type II (β = 0.20) errors. In addition, 108 primiparous cows enrolled in the CCFA had pregnancy to first postpartum AI results, and this sample size was sufficient (α = 0.05, β = 0.20) to detect a 12.75-percentage-unit difference in pregnancy to first AI between AMP and CCFA when the pregnancy to first AI of AMP treated cows is 25%. When the interaction between treatment and severity of the disease was used, the smallest sample size was 96 cows with puerperal metritis in the CCFA treatment, and it was sufficient (α = 0.05, β = 0.20) to detect a 12-percentage-unit difference in cure on d 11 after diagnosis (e.g., CCFA = 80 vs. AMP = 68%).

Data were analyzed using SAS version 9.3 (SAS Institute Inc., Raleigh, NC). In preliminary data analyses, we determined that the interaction between treatment and severity of metritis did not affect any of the outcomes. Therefore, we analyzed the data of cows enrolled in the AMP, CCFA, and HTY treatments without including severity of disease. When analyzing outcomes that were measured only in AMP- and CCFA-treated cows (e.g., RT), however, we included severity of metritis and the interaction between treatment and severity of metritis in the model. In all models, treatment (AMP, CCFA, HTY), parity (primiparous vs. multiparous), farm, calving disorders (yes vs. no), and interactions between treatment and parity were included as fixed effects. For analysis of resumption of cyclicity we included in the model the method through which resumption of cyclicity was determined. For pregnancy per AI and pregnancy loss, the models also included code of insemination (estrus vs. timed AI). Treatment was forced in all models, and the other independent variables and interactions were removed by backward elimination when P > 0.10. To determine the effects of treatment (AMP vs. CCFA) and the effect of metritis (AMP + CCFA vs. HTY) on the outcomes of interest, we used orthogonal contrasts.

Binomial outcomes (cure, PVD, cytological endometritis, pregnancy and pregnancy loss, removal from the herd) were analyzed by logistic regression using the LOGISTIC procedure. The hazard of removal from the herd (death and sale) and pregnancy within 305 DIM were analyzed via Cox proportional hazards regression, using the PHREG procedure. The differences in the intervals from calving to removal from the herd and from calving to pregnancy were analyzed by the Kaplan-Meier survival analysis using the LIFETEST procedure. When analyzing the hazard of pregnancy, cows were censored when they died, were sold, were deemed not eligible for AI, or did not conceive by 305 DIM.

Continuous variables were analyzed by ANOVA. Outcomes that did not have a normal distribution of the residuals (concentrations of haptoglobin, BHB, and NEFA) were log-transformed, but data were back-
transformed for presentation. Rectal temperature on d 0 and 11 and concentrations of metabolites and haptoglobin on d 0 and 14 were analyzed individually using the GLM procedure. Rectal temperatures between d 1 and 6 were analyzed as repeated measures, and the structure of covariance was chosen according to the Bayesian information criterion. For the analysis of the effect of treatment on RT, temperature on d 0 was used as a covariate, and the 3-way interaction among treatment, severity, and day was included in the model. We analyzed the concentrations of metabolites and haptoglobin from d 1 to 8 as repeated measures using the ante-dependence structure of covariance. We used their respective concentrations on d 0 as covariates. For the analysis of milk yield, the structure of covariance was chosen according to the Bayesian information criterion, week was used as the repeated measure, and the 3-way interaction among treatment, parity, and week was included in the model.

For the analyses of behavior data (feeding time, rumination time, activity, and resting time), day relative to enrollment was used as the repeated measure. We used each cow’s respective averages from d −7 to −1 relative to diagnosis as covariates, excluding prepurchase data. The pre- and postdiagnosis periods were analyzed separately. The structure of covariance was chosen according to the Bayesian information criterion. For all repeated-measure analyses, we used cow as the random variable and nested it within treatment.

Statistical significance was declared at \( P \leq 0.05 \), and a tendency was declared at \( 0.05 < P \leq 0.10 \).

**RESULTS**

We examined 2,424 cows, and the incidences of metritis and puerperal metritis were 19.0% (n = 460) and 8.1% (n = 197), respectively. Thirty-nine cows were not enrolled in the experiment for the following reasons: nonfunctional quarters (n = 3), gestation length <260 d (n = 11), antimicrobial treatment between calving and diagnosis (n = 13), left displacement of the abomasum on the day of diagnosis (n = 3), severe lameness (n = 3), mastitis on the day of diagnosis (n = 4), recumbent for more than 2 d before enrollment (n = 1), and suspect of clostridiosis (n = 1). Therefore, we enrolled 618 cows in the experiment. Five cows were not included in the statistical analyses because of incomplete treatments (CCFA: 2 cows were sold, and 2 cows were suspect of clostridiosis; AMP: 1 accidental death during administration of oral drench).

The incidence of puerperal metritis (AMP = 33.1%, CCFA = 33.2%), male (AMP = 43.4%, CCFA = 44.2%), retained fetal membranes (AMP = 26.4%, CCFA = 23.3%), and vaginal laceration (AMP = 52.9%, CCFA = 51.6%) were not \( P \geq 0.40 \) different between treatments. The interaction between treatment and parity, however, was \( P = 0.04 \) associated with incidence of calving disorders (AMP: primiparous = 32.8%, multiparous = 19.2%; CCFA: primiparous = 27.0%, multiparous = 28.3%).

Throughout the experiment, the THI of the nonsalable-milk and postpartum pens were not \( P = 0.14 \) different, regardless of herd (farm A: nonsalable-milk pen = 80.4 ± 0.7, postpartum pen = 81.2 ± 0.7; farm B: nonsalable-milk pen = 82.6 ± 0.7, postpartum pen = 80.9 ± 0.7).

**Effects of Treatment on Rectal Temperature, Cure, and Uterine Health**

At enrollment, RT of cows enrolled in the AMP and CCFA treatment did not \( P = 0.94 \) differ (Figure 1). Cows enrolled in the AMP treatment had \( P < 0.01 \) greater RT from d 1 to 6 compared with cows treated with CCFA. On d 11, we detected a tendency \( P = 0.09 \) for the interaction between treatment and severity of metritis to affect RT because, among cows enrolled in the AMP treatment, those originally diagnosed with puerperal metritis had \( P < 0.01 \) greater RT than those that had been diagnosed with metritis. Meanwhile, among cows enrolled in the CCFA treatment, we did not \( P = 0.30 \) detect a difference in RT according to severity of metritis.

Treatment did not \( P \geq 0.35 \) affect the likelihood of cure (absence of escape therapy by d 11 + VD <5, absence of escape therapy by d 11 + VD <5 + RT <39.5°C) at 4, 6, and 11 d after diagnosis (Table 2). According to VD <5, cows diagnosed with puerperal metritis were \( P < 0.01 \) less likely to be cured at 4 (18.5 ± 2.9 vs. 33.4 ± 2.8%), 6 (32.0 ± 3.5 vs. 49.8 ± 2.9%), and 11 (63.0 ± 3.7 vs. 75.6 ± 2.3%) d after diagnosis compared with cows with metritis. Similarly, according to VD <5 and RT <39.5°C, cows diagnosed with puerperal metritis were \( P < 0.01 \) less likely to be cured at 4 (15.5 ± 2.7 vs. 29.2 ± 2.7%), 6 (26.7 ± 3.3 vs. 46.3 ± 2.9%), and 11 (58.4 ± 3.8 vs. 72.3 ± 2.3%) d after diagnosis. Treatment did not \( P = 0.11 \) affect the percentage of cows that were treated with antimicrobial escape therapy (Table 2). Regardless of treatment, cows diagnosed with puerperal metritis were \( P < 0.01 \) more likely to receive antimicrobial escape therapy by d 11 compared with cows diagnosed with metritis (2.7 ± 1.5 vs. 0.7 ± 0.4%).

Cows treated with AMP were \( P < 0.01 \) more likely to be diagnosed with PVD than cows treated with CCFA, whereas HTY cows were \( P < 0.01 \) less likely
to be diagnosed with PVD than cows diagnosed with metritis (Table 3). Similarly, cows treated with AMP tended \((P = 0.09)\) to have greater incidence of cytological endometritis compared with cows treated with CCFA, and HTY cows had \((P < 0.01)\) the smallest incidence of cytological endometritis (Table 3). Among cows diagnosed with cytological endometritis, the average number of PMNL per 200 cells in the uterine cytology were 78.8 ± 13.1 and 69.3 ± 14.4 among multiparous cows enrolled in the AMP and CCFA treatments, respectively, and 54.6 ± 17.4 and 92.1 ± 19.8 for primiparous cows enrolled in the AMP and CCFA treatments, respectively.

**Effects of Treatment on Concentrations of Haptoglobin and Metabolites and Body Condition Score**

Concentration of haptoglobin on d 0 tended to be greater \((P = 0.09)\) for cows enrolled in the CCFA treatment compared with cows enrolled in the AMP treatment (Figure 2). In addition, cows diagnosed with metritis had \((P < 0.01)\) greater concentration of haptoglobin than HTY cows (Figure 2). Concentrations of haptoglobin from d 1 to 8 were not \((P = 1.00)\) different between cows treated with CCFA and AMP, whereas HTY cows had \((P < 0.01)\) lower concentration of haptoglobin from d 1 to 8 compared with cows diagnosed with metritis (Figure 2). On d 14, cows treated with CCFA had \((P = 0.03)\) greater concentration of haptoglobin than cows treated with AMP, and HTY cows had \((P < 0.01)\) lower concentration of haptoglobin compared with cows diagnosed with metritis (Figure 2).

At enrollment, concentration of glucose was \((P = 0.04)\) greater for cows enrolled in the CCFA treatment compared with cows enrolled in the AMP treatment, but the concentration of glucose of HTY cows was not \((P = 0.59)\) different than concentration of glucose of cows diagnosed with metritis (Figure 3). From d 1 to 8, concentrations of glucose were \((P < 0.01)\) greater for cows treated with AMP compared with cows treated with CCFA, whereas HTY cows had \((P < 0.01)\) reduced concentration of glucose compared with cows diagnosed with metritis (Figure 3). The interaction between treatment and parity affected \((P < 0.01)\) the concentration of glucose from d 1 to 8. Among primiparous cows, those treated with AMP (69.8 ± 1.3 mg/dL) had \((P = 0.04)\) and tended to have \((P = 0.09)\) greater glucose concentration compared with cows treated with CCFA (66.6 ± 1.1 mg/dL) and HTY cows (58.1 ± 1.0 mg/dL), respectively. Among multiparous cows, those treated with AMP (66.2 ± 1.1 mg/dL) had \((P < 0.01)\) the greatest glucose concentration, followed by those treated with CCFA (66.6 ± 1.2 mg/dL) and HTY cows (67.1 ± 1.3 mg/dL), respectively, but CCFA and HTY treatments did not \((P = 0.72)\) differ. Among multiparous cows, those treated with AMP (66.2 ± 1.1 mg/dL) had \((P < 0.01)\) the greatest glucose concentration, followed by those treated with CCFA (62.6 ± 1.1 mg/dL) and HTY cows (58.1 ± 1.0 mg/dL), respectively. On d 14, we did not \((P = 0.41)\) detect an effect of treatment on glucose concentration (Figure 3).

Concentration of NEFA on d 0 tended \((P = 0.09)\) to be greater for cows treated with CCFA compared with cows treated with AMP (Figure 4). As expected, cows diagnosed with metritis had \((P < 0.01)\) greater concentration of NEFA at enrollment than HTY cows (Figure 4). The interaction between treatment and day affected \((P < 0.01)\) the concentration of NEFA from d 1 to 8 (Figure 4). On d 1, cows enrolled in the AMP treat-
ment had \((P < 0.01)\) greater concentration of NEFA than cows enrolled in the CCFA treatment and HTY cows, whereas from d 6 to 8 HTY cows had \((P \leq 0.02)\) greater NEFA concentration than cows enrolled in the AMP and CCFA treatments. We did not \((P = 0.49)\) detect an effect of treatment on NEFA concentration on d 14 (Figure 4).

On d 0, concentration of BHB was not \((P = 0.79)\) different among treatments (Figure 5). From d 1 to 8, the interaction between treatment and day affected \((P < 0.01)\) concentration of BHB (Figure 5). From d 2 to 7, cows treated with CCFA had \((P \leq 0.01)\) greater BHB concentration than cows enrolled in the AMP treatment. Concentration of BHB from d 2 to 8 was \((P < 0.01)\) greater for HTY cows than for those enrolled in the AMP treatment, whereas from d 6 to 8 HTY cows had \((P < 0.01)\) greater concentration of BHB than cows treated with CCFA. On d 14, cows treated with CCFA had \((P < 0.01)\) lower concentration of BHB than cows treated with AMP; however, concentration of BHB was not different \((P = 0.25)\) between HTY cows and cows diagnosed with metritis (Figure 5).

Body condition scores at 1 ± 3, 35 ± 3, and 63 ± 3 DIM did not \((P \geq 0.50)\) differ between cows enrolled in the AMP and CCFA treatments (Table 3). Healthy cows tended \((P = 0.06)\) to have and had \((P < 0.01)\) greater BCS at 1 and 35 DIM, respectively, than cows with metritis. Nonetheless, BCS of HTY cows at 63 ± 3 DIM did not \((P = 0.14)\) differ from the BCS of cows with metritis (Table 3).

### Table 2. Effects of treatment on cure 4, 6, and 11 d after enrollment

<table>
<thead>
<tr>
<th>Variable, % ± SEM (n)</th>
<th>Treatment (^1)</th>
<th>P-value (^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AMP</td>
<td>CCFA</td>
</tr>
<tr>
<td>Cure based on VD (^3) &lt;5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 4 after enrollment</td>
<td>25.6 ± 2.9 (307)</td>
<td>24.9 ± 2.9 (306)</td>
</tr>
<tr>
<td>d 6 after enrollment</td>
<td>40.3 ± 3.3 (307)</td>
<td>40.4 ± 3.2 (306)</td>
</tr>
<tr>
<td>d 11 after enrollment</td>
<td>68.6 ± 3.0 (302)</td>
<td>67.6 ± 3.0 (304)</td>
</tr>
<tr>
<td>Cure based on VD &lt;5 and RT (^4) &lt;39.5°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 4 after enrollment</td>
<td>21.7 ± 2.7 (307)</td>
<td>21.5 ± 2.7 (306)</td>
</tr>
<tr>
<td>d 6 after enrollment</td>
<td>33.9 ± 3.1 (307)</td>
<td>37.5 ± 3.2 (306)</td>
</tr>
<tr>
<td>d 11 after enrollment</td>
<td>64.6 ± 3.1 (302)</td>
<td>63.5 ± 3.1 (304)</td>
</tr>
<tr>
<td>Cows that received antimicrobial escape therapy between d 1 and 10</td>
<td>0.92 ± 0.57 (307)</td>
<td>2.06 ± 1.10 (306)</td>
</tr>
</tbody>
</table>

\(^1\)Cows diagnosed with metritis were randomly assigned according to severity of disease [metritis: fetid, watery, red or brown uterine discharge within 21 DIM (VD = 5); puerperal metritis: VD = 5 and rectal temperature ≥39.5°C] and parity to receive 11 mg/kg of ampicillin (AMP) once daily for 5 d or 6.6 mg/kg of ceftiofur crystalline free acid (CCFA) twice, 72 h apart.

\(^2\)TRT = treatment.

\(^3\)VD = vaginal discharge.

\(^4\)RT = rectal temperature.

#### Rumination, Activity, Feeding, and Resting Times Relative to Enrollment in the Experiment

During the pre-diagnosis period, we observed that the interaction between treatment and day was \((P < 0.01)\) associated with resting time (Figure 6A). Healthy cows had \((P < 0.01)\) lower resting time, from d −6 to −1, than cows enrolled in the AMP treatment and, from d −5 to −1, than cows enrolled in the CCFA treatment. Similarly, we observed that the interaction between treatment and day affected \((P < 0.01)\) resting time after diagnosis (Figure 6A). Cows treated with AMP had \((P \leq 0.05)\) lower resting time compared with cows treated with CCFA from d 2 to 5, whereas HTY cows had \((P \leq 0.04)\) lower resting time than cows treated with AMP from d 0 to 3 and cows treated with CCFA from d 0 to 6.

The interaction between treatment and day was \((P < 0.01)\) associated with pre-diagnosis feeding time (Figure 6B). Feeding time was not \((P = 0.55)\) different between cows enrolled in the AMP and CCFA treatments. On d −6 and from d −4 to −1, HTY cows had \((P \leq 0.05)\) greater feeding time compared with cows diagnosed with metritis, and, on d −5, they tended \((P = 0.06)\) to have greater feeding time than cows enrolled in the CCFA treatment. During the period after diagnosis, cows treated with CCFA had \((P = 0.05)\) greater feeding time than cows treated with AMP, whereas HTY cows had \((P < 0.01)\) greater feeding time compared with cows diagnosed with metritis (Figure 6B).
The interaction between treatment and day was \((P < 0.01)\) associated with pre-diagnosis rumination time (Figure 6C). Healthy cows had greater rumination time from \(d \, -4\) to \(-1\) and from \(d \, -3\) to \(-1\) than cows enrolled in the AMP and CCFA treatments, respectively. The interaction between treatment and day affected \((P < 0.01)\) rumination time after diagnosis. Cows treated with CCFA had \((P = 0.01)\) greater rumination time on \(d \, 7\) compared with cows treated with AMP, whereas HTY cows had \((P \leq 0.01)\) greater rumination time from \(d \, 0\) to 6 and from \(d \, 0\) to 8 compared with CCFA- and AMP-treated cows, respectively.

During the pre-diagnosis period, activity did not \((P = 0.92)\) differ between cows enrolled in the CCFA and AMP treatments. Healthy cows, however, had \((P < 0.01)\) greater activity during the entire pre-diagnosis period than cows diagnosed with metritis (Figure 6D). The interaction between treatment and day affected \((P < 0.01)\) activity following diagnosis. Cows treated with CCFA had \((P \leq 0.04)\) reduced activity on \(d \, 0, 1, 7,\) and 8 compared with cows treated with AMP. Furthermore, HTY cows had \((P \leq 0.05)\) greater activity on \(d \, 3\) and 4 compared with cows diagnosed with metritis (Figure 6D). Additionally, HTY cows tended \((P \leq 0.09)\) to have greater activity on \(d \, 0\) and 1 than cows treated with CCFA, and activity of HTY cows on \(d \, 3\) and 4 was \((P \leq 0.05)\) to be lower compared with cows treated with AMP (Figure 6D).

### Reproductive Performance, Milk Production, and Culling

Treatment did not \((P = 0.33)\) affect the likelihood of resumption of estrous cycles (Table 3). In addition, the likelihood of pregnancy after the first postpartum AI \((P \geq 0.16)\) and of pregnancy loss between 40 and 60 d of gestation \((P = 0.75)\) were not affected by treatment. We detected no \((P \geq 0.34)\) differences between the CCFA and AMP treatments regarding the likelihood of pregnancy at 40 and 60 d after the second postpartum AI.
AI. Healthy cows, however, were ($P \leq 0.05$) more likely to be pregnant at 40 and 60 d after the second postpartum AI than cows diagnosed with clinical health disorder ($n = 122$). Day 0: treatment $P < 0.01$, AMP vs. CCFA $P = 0.09$, AMP+CCFA vs. HTY $P < 0.01$. Days 1 to 8: treatment $P < 0.01$, day $P < 0.01$, AMP vs. CCFA $P = 1.00$, AMP+CCFA vs. HTY $P < 0.01$. Day 14: treatment $P < 0.01$, AMP vs. CCFA $P = 0.03$, AMP+CCFA vs. HTY $P < 0.01$. Values are presented as LSM ± SEM.

The hazard of pregnancy was ($P < 0.01$) affected by the interaction between treatment and parity. Among primiparous cows, we determined that cows treated with CCFA [adjusted hazard ratio (AHR) = 0.92; 95% CI = 0.67, 1.27] had ($P = 0.01$) reduced hazard of pregnancy than those treated with AMP (AHR = 1.40; 95% CI = 1.03, 1.90), but the hazard of pregnancy was not ($P = 0.35$) different between HTY cows (referent) and cows diagnosed with metritis. The Wilcoxon test of equality determined a tendency ($P = 0.07$) for treatment to affect the interval from calving to pregnancy among primiparous cows (Figure 7A). The mean (±SEM) and median intervals from calving to pregnancy were 145.0 ± 7.7 and 115 d, 169.4 ± 8.6 and 141 d, and 159.4 ± 6.9 and 136 d for cows in the AMP, CCFA, and HTY treatments, respectively. Among multiparous cows, the hazard of pregnancy was not ($P = 0.96$) different between the CCFA (AHR = 0.67; 95% CI = 0.53, 0.85) and AMP (AHR = 0.70; 95% CI = 0.53, 0.84) treatments, but HTY cows (referent) had ($P < 0.01$) greater hazard of pregnancy than cows diagnosed with metritis. As such, the interval from calving to the

Figure 2. Effect of treatment on haptoglobin concentration. Day 0 = day of diagnosis. AMP = 11 mg/kg of ampicillin trihydrate once daily for 5 d ($n = 95$). CCFA = 6.6 mg/kg of ceftiofur crystalline free acid, twice, 72 h apart ($n = 106$). HTY = cows not diagnosed with clinical health disorder ($n = 122$). Day 0: treatment $P < 0.01$, AMP vs. CCFA $P = 0.09$, AMP+CCFA vs. HTY $P < 0.01$. Days 1 to 8: treatment $P < 0.01$, day $P < 0.01$, AMP vs. CCFA $P = 1.00$, AMP+CCFA vs. HTY $P < 0.01$. Day 14: treatment $P < 0.01$, AMP vs. CCFA $P = 0.03$, AMP+CCFA vs. HTY $P < 0.01$. Values are presented as LSM ± SEM.

Figure 3. Effect of treatment on glucose concentrations. Day 0 = day of diagnosis. AMP = 11 mg/kg of ampicillin trihydrate once daily for 5 d ($n = 95$). CCFA = 6.6 mg/kg of ceftiofur crystalline free acid, twice, 72 h apart ($n = 106$). HTY = cows not diagnosed with clinical health disorder ($n = 122$). Day 0: treatment $P = 0.10$, AMP vs. CCFA $P = 0.04$, AMP+CCFA vs. HTY $P = 0.59$. Days 1 to 8: treatment $P < 0.01$, day $P < 0.01$, AMP vs. CCFA $P < 0.01$, AMP+CCFA vs. HTY $P < 0.01$. Day 14: treatment $P = 0.41$, AMP vs. CCFA $P = 0.20$, AMP+CCFA vs. HTY $P = 0.73$. Values are presented as LSM ± SEM.

Figure 4. Effect of treatment on concentration of nonesterified fatty acid (NEFA). Day 0 = day of diagnosis. AMP = 11 mg/kg of ampicillin trihydrate once daily for 5 d ($n = 95$). CCFA = 6.6 mg/kg of ceftiofur crystalline free acid, twice, 72 h apart ($n = 106$). HTY = cows not diagnosed with clinical health disorder ($n = 122$). Day 0: treatment $P < 0.01$, AMP vs. CCFA $P = 0.09$, AMP+CCFA vs. HTY $P < 0.01$. Days 1 to 8: treatment $P = 0.02$, day $P < 0.01$, treatment $\times$ day $P < 0.01$, AMP vs. CCFA $P = 0.17$, AMP+CCFA vs. HTY $P = 0.01$. Day 14: treatment $P = 0.49$, AMP vs. CCFA $P = 0.54$, AMP+CCFA vs. HTY $P = 0.30$. Pairwise comparisons with $P \leq 0.05$: *AMP vs. CCFA; †HTY vs. AMP; and ‡HTY vs. CCFA. Values are presented as LSM ± SEM.
establishment of pregnancy was \( (P < 0.01) \) affected by treatment (Figure 7B). The mean (±SEM) and median intervals from calving to pregnancy were 185.4 ± 7.6 and 170 d, 189.3 ± 7.6 and 174 d, and 150.4 ± 4.0 and 126 d for cows in the AMP, CCFA, and HTY treatments, respectively.

The interaction between treatment, parity, and week relative to calving affected \( (P < 0.01) \) weekly milk yield from calving to 14 wk postpartum (Figure 8). Among primiparous cows, milk yield of HTY cows was \( (P < 0.02) \) and tended to be \( (P = 0.06) \) greater from wk 1 to 5 and on wk 6, respectively, than cows treated with AMP. In addition, HTY primiparous cows had \( (P < 0.04) \) and tended to have \( (P < 0.10) \) greater milk yield from wk 1 to 6 and from wk 7 to 10, respectively, than primiparous cows treated with CCFA. Primiparous cows enrolled in the AMP, CCFA, and HTY treatments had mean (±SEM) milk yield from calving to 14 wk postpartum of 32.6 ± 0.8, 32.3 ± 0.8, and 34.4 ± 0.7 kg/d, respectively. Healthy multiparous cows had \( (P < 0.05) \) and tended \( (P < 0.04) \) and \( (P < 0.02) \) and tended to be \( (P = 0.06) \) greater from wk 1 to 6 and on wk 11, respectively, than cows treated with CCFA. The mean (±SEM) milk yield from calving to 14 wk postpartum of multiparous cows enrolled in the AMP, CCFA, and HTY treatments were 43.8 ± 0.7, 43.1 ± 0.6, and 46.3 ± 0.7 kg/d, respectively.

A greater proportion of cows diagnosed with metritis left the herd within 305 DIM compared with HTY cows \( (P < 0.01) \). Among cows diagnosed with metritis, however, treatment did not affect the proportion of cows that left the herd by 305 DIM \( (P = 0.79) \). The hazard of removal by 305 DIM was \( (P = 0.01) \) affected by treatment. According to the orthogonal contrast, the hazard of removal by 305 DIM did not \( (P = 0.79) \) differ between cows treated with AMP \( (AHR = 1.41; \text{95% CI} = 1.07, 1.88) \) and CCFA \( (AHR = 1.47; \text{95% CI} = 1.11, 1.95) \), whereas HTY cows (referent) had \( (P < 0.01) \) reduced hazard of removal compared with cows diagnosed with metritis. The mean (±SEM) intervals from calving to removal were 257.1 ± 5.1, 276.4 ± 2.8, and 276.4 ± 2.8 d for cows enrolled in the AMP, CCFA, and HTY treatments, respectively.

**DISCUSSION**

Ceftiofur crystalline free acid and AMP are drugs commonly used in the United States for the treatment of metritis, despite the fact that the latter is not labeled for such use. The extralabel use of antimicrobials requires justification by the prescribing veterinarian based on the Animal Medicinal Drug Use Clarification Act of 1994 guidelines (FDA, 2012). The selection of extralabel use of penicillin and ampicillin to treat metritis by farmers results from their perception that these drugs are more efficacious than ceftiofur (Espadamala et al., 2018). The percentage of cows cured on d 11 was approximately 70%, which is in accordance with a previous report (Lima et al., 2014). In contrast with our experiment, Lima et al. (2014) used ceftiofur hydrochloride to treat cows diagnosed with metritis. Both formulations of ceftiofur are metabolized into des-fluroceftiofur as the primary metabolite, but CCFA is a cottonseed oil–based suspension with caprylic and capric triglycerides, which results in the slow and sustained release of the drug over time (Fultz et al., 2013). Therefore, treatment with ceftiofur hydrochloride must be repeated at 24-h intervals for 5 d, whereas CCFA must be repeated once, 72 h after the first treatment. Regardless of the severity of metritis, cows treated with AMP had higher RT than cows treated with CCFA, from 1 to 6 d after diagnosis. Similar to our findings, Lima et al. (2014) demonstrated that, regardless of the severity of the disease, cows treated with AMP had higher RT in the first days of treatment compared with ceftiofur-treated cows. The possible effects of a reduction in RT by 0.1 to 0.2°C are unclear, but according to our results, they are likely minor.

![Figure 5. Effect of treatment on concentration of BHB. AMP = 11 mg/kg of ampicillin trihydrate once daily for 5 d (n = 95). CCFA = 6.6 mg/kg of ceftiofur crystalline free acid, twice, 72 h apart (n = 106). HTY = cows not diagnosed with clinical health disorder (n = 122). Day 0: treatment F = 0.79, AMP vs. CCFA F = 0.57, MET vs. HTY F = 0.71. Days 1 to 8: treatment P < 0.01, day P < 0.01, treatment × day P < 0.01, AMP vs. CCFA P < 0.01, AMP+CCFA vs. HTY P < 0.01. Day 14: treatment P = 0.01, AMP vs. CCFA P < 0.01, AMP+CCFA vs. HTY P = 0.25. Pairwise comparisons with P ≤ 0.05: *AMP vs. CCFA; †HTY vs. AMP; and ‡HTY vs. CCFA. Values are presented as LSM ± SEM.](image-url)
In the current experiment, regardless of the type of antimicrobial used, the treatment of cows diagnosed with metritis was associated with a decrease in concentrations of haptoglobin over time. The concentrations of haptoglobin of cows diagnosed with puerperal metritis and treated with penicillin, oxytetracycline, or ceftiofur declined during the 5-d treatment period, regardless of the antimicrobial used (Smith et al., 1998). We observed that HTY cows had lower concentrations of haptoglobin from d 0 to 14 compared with cows diagnosed with metritis, in accordance with the findings of Huzzey et al. (2009). Although the concentration of haptoglobin from 1 to 8 d after the diagnosis of metritis was nearly 3 times greater than the concentration of haptoglobin in HTY cows, on d 14 after diagnosis, the difference in concentration of haptoglobin between cows with metritis and HTY cows was minute.

The concentrations of metabolites and behavior data from the current experiment shed some light on the challenges faced by cows diagnosed with acute infectious diseases early postpartum. The longer resting time and reduced feeding and activity times pre-diagnosis among cows diagnosed with metritis may be a consequence of cytokine-induced sickness behavior (Dantzer and Kelley, 2007). During the inflammatory process, proinflammatory cytokines (e.g., IL-6 and -8, tumor necrosis factor α) modify the behavior of the host through alterations in tryptophan metabolism.
and serotonin neurotransmission (Dantzer and Kelley, 2007), causing depression-like behaviors. Furthermore, Hart (1988) postulated that an animal that spends less time moving and searching for feed saves energy for the increased metabolic rate induced by inflammation and reduces its exposure to predators. Cows treated with AMP had the greatest concentrations of glucose from d 1 to 8, followed by cows enrolled in the CCFA and HTY treatments, respectively. Conversely, cows in the AMP treatment had the highest NEFA concentration on d 1, whereas, from d 6 to 8, HTY cows had the highest NEFA concentrations. On d 2, cows treated with AMP had the lowest BHB concentration, whereas, on d 6 and 7, HTY cows had the highest BHB concentration, followed by CCFA- and AMP-treated cows, respectively. We also demonstrated that feeding time of cows in the AMP treatment was reduced compared with the feeding time of cows treated with CCFA. In the current experiment we did not assess the effects of treatments on DMI, daily milk yield during treatment, or energy balance. We speculate, however, that the AMP treatment, which involves the movement of cows with metritis to a nonsalable-milk pen, may have affected these outcomes, resulting in the observed changes in metabolites. The greater postdiagnosis activity of cows treated with AMP, compared with HTY cows and
cows treated with CCFA, is likely a consequence of the movement of the former to the nonsalable-milk pen. It is worth noting that the maximum difference in activity observed within a day between AMP and CCFA was 31.8 min, which may be biologically negligible. Healthy cows had lower resting time than cows diagnosed with metritis. Similar to our findings, Neave et al. (2018) demonstrated that cows diagnosed with metritis spent more time lying down than healthy cows during wk −2 and −1 pre-diagnosis. Cows treated with CCFA had longer feeding time during the entire postdiagnosis period compared with cows treated with AMP, but the maximum difference between treatments was 12.6 min/d. On d 7 after diagnosis, cows treated with CCFA had longer rumination time than cows treated with AMP. Within a 2-h interval, rumination is negatively correlated with DMI, but, over time, decreased DMI is associated with reduced daily rumination time (Schirmann et al., 2013). A normal daily variation in rumination time is assumed to be approximately 10%, but a large decrease in rumination time is commonly interpreted as presence of discomfort (Beauchemin, 2018). Although one may argue that cows treated with AMP had greater discomfort than cows treated with CCFA, particularly on the day of pen movement, AMP treatment resulted in 5 to 10% decreases in resting, feeding, and rumination times, which may not be biologically relevant. To conclude, we demonstrated that metritis has a profound effect on inflammatory response and feeding and rumination times, and that these changes could explain the significant differences in concentration of metabolites between HTY cows and cows with metritis. Future experiments should aim to determine in what circumstances treatment of cows diagnosed with postpartum infectious diseases with antimicrobials are necessary to improve these outcomes.

Cows treated with AMP were more likely to have PVD and tended to be more likely to have cytological endometritis than cows treated with CCFA. Our results were unexpected because Lima et al. (2014) reported a higher prevalence of PVD for cows treated with ceftiofur than for cows treated with AMP and no difference between them regarding the prevalence of cytological endometritis. In our experiment, the prevalence of PVD and cytological endometritis were greater than those reported by Lima et al. (2014). Presence of pus in the uterus is correlated with the presence of uterine pathogenic bacteria (LeBlanc, 2012); thus, it was not surprising that HTY cows were less likely to have PVD and cytological endometritis than cows diagnosed with metritis. The finding that metritis influenced the reproductive performance of lactating dairy cows was expected, given that the effects of uterine disease on reproduction is well established (Fourichon et al., 2000; LeBlanc, 2008). Surprisingly, primiparous cows treated with AMP had greater hazard of pregnancy than those treated with CCFA. In addition, primiparous cows treated with AMP had greater hazard of pregnancy than HTY primiparous cows. It is difficult to explain why AMP-treated primiparous cows, which had a greater likelihood of PVD and cytological endometritis, had greater hazard of pregnancy. Differ-
ences in the reproductive outcomes of cows that present pathological and physiological inflammation depend on the severity, timing, and duration of the inflammatory process (LeBlanc, 2012). Some degree of inflammation is a common adaptive feature after calving, given that some level of inflammation is actually favorable to milk production and for adaption during the transition from nonlactating to lactating status (Farney et al., 2013). Thus, debate still exists regarding the amount of inflammation needed for physiological processes to occur normally and the amount that is detrimental for performance (Abuelo et al., 2015). In the subset of cows used for endometrial cytology, primiparous cows treated with AMP (54.6 ± 17.4 PMNL/200 cells) had numerically fewer PMNL than primiparous cows treated with CCFA (92.1 ± 19.8 PMNL/200 cells). We speculate that if, in fact, primiparous cows treated with CCFA had an exacerbated inflammatory response compared with AMP-treated cows, this could have had a long-lasting effect on uterine environment that could have reduced hazard of pregnancy. Another possible hypothesis to explain the differences in hazard of pregnancy between primiparous AMP- and CCFA-treated cows is the possible difference in bacterial population in their reproductive tract. Treatment of metritic cows with AMP was more effective against Porphyromonas, which has been associated with metritis (Cunha et al., 2018) and necrotic vulvovaginitis (Elad et al., 2004), than ceftiofur (Jeon et al., 2018). In addition, nonuterine diseases may interfere with reproductive performance (Ribeiro et al., 2016). When we analyzed the incidence of mastitis from calving until first breeding among cows enrolled in the current experiment, we did not detect a difference between cows treated with AMP and CCFA (data not shown). The collaborating herds did not systematically examine cows for lameness, ketosis, subclinical hypocalcemia, and other nonuterine disorders; thus, it is not possible to rule out that primiparous cows treated with CCFA had greater incidence of nonuterine diseases than those treated with AMP. The greater hazard of pregnancy of primiparous cows treated with AMP compared with those treated with CCFA and HTY cows could be a spurious finding. This is particularly true when we take into consideration that no differences in hazard of pregnancy were detected between multiparous cows treated with CCFA and AMP and that these cows had lower hazard of pregnancy than HTY cows, which is more consistent with the literature (Sheldon and Dobson, 2004; Azawi, 2008; Dubuc et al., 2010b).

Milk yield within 14 wk postpartum was not different between cows treated with AMP and CCFA, but their milk yield was reduced compared with HTY cows. In light of the lack of difference in milk between CCFA and AMP, we conclude that the changes in feeding and resting time associated with a treatment strategy that requires movement of the cow to a nonsalable-milk pen are minute and, likely, biologically unimportant. Other experiments should evaluate the effects of pen movement on behavior and milk yield of cows diagnosed with infectious disease and treated with the same antimicrobial. Lima et al. (2019) reported that cows treated with AMP had greater milk yield than cows treated with ceftiofur and that healthy cows had greater milk yield compared with cows diagnosed with metritis. Our results disagree with those of some other researchers, who did not report an association between metritis and milk yield (Bartlett et al., 1986) but are in agreement with others, who reported a loss of 2.7 kg/day for cows with metritis (Rajala and Gröhn, 1998). Furthermore, similarly to a previous experiment (Dubuc et al., 2010a), we did not observe an effect of antimicrobial treatment on culling within 305 DIM. In addition, metritis was not associated with culling within 305 DIM, in accord with Dubuc et al. (2011).

CONCLUSIONS

Although the reduced rectal temperature and improved uterine health of cows treated with CCFA compared with those treated with AMP would lead us to expect improved reproduction among the former, primiparous cows treated with AMP had greater hazard of pregnancy than those treated with CCFA and HTY cows. Among multiparous cows, however, we did not observe differences in hazard of pregnancy between cows treated with AMP and CCFA, and both had reduced hazard of pregnancy compared with HTY cows. The mechanisms of action of AMP to improve the hazard of pregnancy among primiparous cows should be more carefully investigated, because it may lead to new discoveries on how to improve the effectiveness of metritis treatments. In the current experiment, metritis was associated with abrupt changes in behavior commonly associated with welfare. Regardless of antimicrobial treatment, behavioral responses quickly improved following diagnosis and treatment. Additional experiments that compare the behavior and welfare of treated and untreated cows diagnosed with metritis and other acute diseases are highly encouraged.

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