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**Ruminant Nutrition**

# Energy restriction and housing of pregnant beef heifers in mud decreases body weight and conceptus free live weight

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## ABSTRACT

Average temperatures in the Midwest, USA are predicted to increase 2–9°C by the end of the century; resulting in muddy pastures for spring calving beef heifers as they enter late gestation. The objective of this study was to evaluate the effects of muddy conditions on heifer body weight (BW), body condition score (BCS), conceptus free live weight (CFLW), and fetal growth when heifers were energy restricted during late gestation. Eighteen Angus heifers ($n$ = 9/treatment) were paired based on initial BW. One heifer from each BW pair was randomly allocated to either the mud (MUD) or control (CON) treatment on day 196 of gestation. Pens in the CON treatment were bedded with wood chips, while pens in the MUD treatment were filled with mud (average depth of 19.5 ± 7.9 cm). Heifers were housed individually and fed the same diet that consisted of a limit-fed total mixed ration from day 196 to 266 of gestation that was formulated to meet 66% of the net energy for maintenance, growth, and gestation requirements. Requirements and the amount of the diet offered were adjusted weekly, and heifers were weighed and sampled for blood metabolites weekly. Data were analyzed as a randomized complete block design with repeated measurements. There was a treatment × day of gestation interaction, such that heifers had similar BW, BCS, and CFLW on day 196 of gestation. By day 266 of gestation; however, heifers in the MUD treatment weighed 43.5 kg less ($P$ < 0.01) and were 1.8 BCS units less ($P$ < 0.01) than heifers in the CON treatment. This is further supported by the treatment × day effects we observed for back fat (BF) and rump fat (RF) thickness, such that the MUD heifers had less BF ($P$ = 0.02) and RF ($P$ < 0.01) by day 266 of gestation. There was a marginally significant difference for gestation length ($P$ = 0.06), such that heifers in the MUD treatment calved approximately 3.1 days before the heifers in the CON treatment. Though heifers in the MUD treatment decreased their BW and CFLW during the treatment period, we did not observe a difference in calf birth weight ($P$ = 0.34), calf plasma IgG concentration ($P$ = 0.37), or calf weaning weight ($P$ = 0.63). Despite heifers in the MUD treatment having greater BW, CFLW, and BCS losses compared with the heifers in the CON treatment, the heifers in the MUD treatment seemed to prioritize fetal growth, as they mobilized their body tissues to meet the energetic demands of pregnancy.

**Key words:** beef heifers, birth weight, mud, net energy requirements

## INTRODUCTION

Pregnant heifers experience an exponential increase in nutrient requirements during the last trimester of gestation. During this time, heifers must continue to grow, provide for the majority of fetal growth, and prepare for parturition and their first lactation (NASEM, 2016). Because of these energetic demands, body condition and thus energy reserves up to calving are critical in pregnant heifers (Whitman, 1975). However, it is common for pregnant heifers in the Midwest, USA to be nutrient restricted during late gestation, as heifers are often managed in a pastoral system that is limiting in both forage quantity and quality (Ciccioli et al., 2003; Meyer et al., 2010; Tipton et al., 2018). There is evidence that late gestation nutrient restriction of the dam can cause intrauterine growth restriction that can result in altered growth and potential long-term consequences such as different disease states later in life (Godfrey and Barker, 2000; Wu et al., 2006). Specifically, in cattle, heifers that were nutrient restricted during the last third of gestation have been reported to have decreased calf birth weights, greater death rates at birth, and decreased weaning weights compared with calves born to heifers that were fed to meet nutrient requirements (Corah et al., 1975). Additionally, poor body condition scores (BCS 3–4) in pregnant heifers are reported to negatively affect calf birth weight, calf vigor, and calf serum immunoglobulin (IgM and $IgG_1$) concentration (Odde, 1988; Spitzer et al., 1995).

Nutrient restriction because of poor forage quantity and quality during gestation can have negative impacts on both the dam and the fetus. The negative effects of nutrient restriction on the dam and fetus may be greater for heifers housed outside where they are unprotected from environmental factors such as cold stress or mud stress (Nickles et al., 2022). Climate models have predicted average daily maximum temperatures in the Midwest, United States to increase from 2 to 9°C by the end of the century, resulting in warmer winters, longer ice-free seasons, longer growing seasons, and an increase in intense rainfall events (Wuebbles and Hayhoe, 2004; Hayhoe et al., 2010). This change in average daily maximum temperature

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is likely to alter the distribution of precipitation events, such that winter and spring precipitation is anticipated to increase by as much as 20% to 30% by the end of the century (Hayhoe et al, 2010). Historically, as winter temperatures continue to increase, more precipitation has been falling as rain and less as snow over the course of the last several decades (Hayhoe et al., 2010). While producers in the upper Midwest have dealt with muddy conditions during the spring calving season in the past, the change in temperature and precipitation events are creating a prolonged environmental stressor for beef cows housed on pasture that is not only occurring during calving but also during much of late gestation.

The dam acts as a buffer between the external environment and the fetus; therefore, it is likely the development of the fetus will only be affected if the demands of the dam and the fetus exceed the nutrient intake of the dam plus her labile tissues (Hight, 1968). When a beef cow is unprotected from the wind and the rain, the hair coat is compromised and not able to properly insulate the animal, thus increasing the animal's metabolic rate to maintain homeothermy (Webster et al., 1970; Webster, 1974; Young, 1983). The NASEM (2016) generally recognizes that mud increases the energy requirements of beef cattle; however, the energetic cost of a muddy environment during late gestation in pregnant heifers is unknown. This caused us to question the prioritization of nutrient use by a pregnant heifer that is energy restricted during late gestation, as well as when a pregnant heifer is energy restricted and housed in muddy environmental conditions. We hypothesized that energy-restricted heifers housed in muddy conditions during late gestation would have increased net energy requirements compared with heifers that were energy restricted but housed in wood chip bedding. We further hypothesized that energy-restricted heifers housed in muddy conditions would mobilize body tissues, decrease body weight and body condition, and produce calves with a decreased birth weight compared with heifers that were housed in wood chip bedding but exposed to the same weather patterns. The objectives of this experiment were to evaluate the prioritization of nutrient use by a pregnant heifer during late gestation that is energy restricted, and to evaluate the effects of muddy environmental conditions when combined with energy restriction on heifer body weight, body condition score, and calf birth weight.

## MATERIALS AND METHODS

All procedures were approved by The Ohio State University Institutional Animal Care and Use Committee (Animal Use Protocol # 2019A00000142).

### Animals, Experimental Design, Treatments

Pregnant Simmental-Angus heifers ($n$ = 9/treatment; initial mean age ± SEM = 638.7 ± 4.3 days; initial mean BW ± SEM = 439.3 ± 3.4 kg; initial mean BCS ± SEM = 6.4 ± 0.1) were used in a randomized complete block design experiment at the Eastern Agricultural Research Station (Caldwell, OH, USA). Heifers were maintained as one herd and treated similarly before the initiation of the study. All heifers entered a fixed time artificial insemination protocol to allow for the same artificial insemination date on May 28, 2020. Pregnancy status was diagnosed using transrectal ultrasonography approximately 30 days after the artificial insemination date. All heifers were confirmed to be pregnant after insemination and had an expected calving date of March 8, 2021 based on the insemination date and a 283-day gestation length. Throughout gestation, heifers were maintained on pasture and were supplemented as necessary to maintain a BCS of 6 to 7 based on the research station's protocol for first-calf heifers.

Before the start of the treatment period, heifers were allowed a two-week adjustment to the experimental diet from day 182 to day 196 of gestation (Table 1). Each week,

**Table 1.** Composition and nutritional profile of the prepartum diet offered to pair fed heifers restricted to 66% of their net energy requirements and either housed in pens filled with mud (MUD; 19.5 ± 7.9 cm) or housed in pens filled with wood chips (CON) from day 196 to day 266 of gestation.

| Item | Day 182[3] to 230 | Day 231 to 244 | Day 245 to 258 | Day 259 to 266 |
|---|---|---|---|---|
| **Composition, as-fed basis** | | | | |
| Beet pulp, % | 7.50 | 7.50 | 7.50 | 7.50 |
| Whole shelled corn, % | 27.00 | 27.00 | 27.00 | 27.00 |
| Corn gluten meal, % | 20.85 | 19.85 | 18.85 | 17.85 |
| Cottonseed hulls, % | 5.00 | 5.00 | 5.00 | 5.00 |
| Chopped hay, % | 38.00 | 39.00 | 40.00 | 41.00 |
| Limestone, % | 1.00 | 1.00 | 1.00 | 1.00 |
| Sodium selenite, % | 0.05 | 0.05 | 0.05 | 0.05 |
| Dicalcium phosphate, % | 0.60 | 0.60 | 0.60 | 0.60 |
| **Nutritional profile[1], dry matter basis** | | | | |
| Net energy for maintenance[2], Mcal/kg | 1.63 | 1.62 | 1.61 | 1.60 |
| Total digestible nutrients, % | 66.17 | 66.05 | 65.94 | 65.82 |
| Neutral detergent fiber, % | 37.30 | 37.64 | 37.97 | 38.30 |
| Acid detergent fiber, % | 25.12 | 25.44 | 25.77 | 26.10 |
| Crude protein, % | 19.96 | 19.39 | 18.82 | 18.24 |

[1]Based on wet chemistry procedures by a commercial laboratory (Rock River Laboratory, Wooster, OH).
[2]Calculations for net energy for maintenance, growth, and gestation of the diet using the feed composition estimates provided by NASEM (2016).
[3]Heifers were given a two-week dietary adjustment period from day 182 to day 196 of gestation when the treatment period began.

the average weight of the 18 heifers was recorded. The maintenance, growth, and gestation requirements were then calculated based on the average weight of all 18 heifers, and the dry matter allowance was calculated such that 66% of those net energy requirements were met and all other nutrient requirements were equaled or exceeded. This dry matter allowance was supplemented with 1.2% of BW of hay based on the mean BW of the 18 heifers. The dry matter allowance of the experimental diet was kept constant throughout the two-week adjustment period; however, the amount of hay was decreased every other day by 0.2% such that by day 14 of the adjustment period, heifers were consuming only the experimental diet that was to be fed during the start of the treatment period on day 196 of gestation.

In December 2020, heifers were ranked and paired on initial BW, and one heifer from each pair was randomly allocated to either the mud (MUD; $n = 9$) or control treatment (CON; $n = 9$). The 18 individual pens were created in the same outdoor lot, were uncovered, and were created away from all buildings to prevent a windbreak effect for certain pens. Before the individual pens were made, the lot was scraped and graded using a skid loader to provide a flat surface. The lot was previously used for holding pens at the research station; therefore, the mud and wood chips were placed on top of the geotextile fabric and stone base. Pens were 4.9 m × 4.9 m, and the 9 pens in the MUD treatment had been filled with soil such that an average mud depth of 19.5 ± 7.9 cm was created. The soil was added from the same area of the research station and the target depth was 30 cm on average at the start of the two-week dietary adjustment period. This allowed for the soil to be in the individual pens for two weeks before the heifers entered the pens and allowed us to ensure that it was truly mud when the heifers started the treatment period on day 196 of gestation. The target depth of 30 cm was based on the depth of mud that heifers were typically subjected to at the research station in previous years. No drainage system was created for the area where the pens were created; however, to maintain the integrity of each treatment pen, the treatments were randomly assigned to row. This allowed for each row of pens to house only one treatment and prevented the mud from entering the CON pens. Furthermore, heifers were randomly assigned to pen within their treatment. The nine pens in the CON treatment had been filled with sawdust and wood chips at approximately the same target depth and were continually bedded as necessary throughout the treatment period as necessary to prevent any mud. While it was not recorded during the treatment period, there were no observations of any consumption of the bedding by the heifers in the CON treatment.

All heifers were housed individually and fed the same diet once daily (Table 1) at approximately 0830 h and dry matter intakes were recorded. Heifers were provided a limit-fed total mixed ration. Each heifer was provided her own feed bunk (1.5 m × 0.7 m) and water trough that was filled daily to provide ad libitum access to water. Once the treatment period started on day 196 of gestation and the heifers were put into their individual pens, the diet was formulated to meet 66% of maintenance, growth, and gestation net energy requirements, and to equal all other maintenance, growth, and gestation nutrient requirements according to the NASEM (2016) based on the CON heifer in each pair. Specifically, heifer maintenance requirements were calculated using the NASEM (2016) equation 11-1 and predicted target weights were calculated using the NASEM (2016) equations 13-15 through 13-25 and assuming a 544 kg mature weight based on the station's mature cow herd weight. Growth requirements for each CON heifer were calculated by adding the average daily gain associated with gravid uterus growth to the predicted shrunk BW average daily gain that was calculated to reach 80% of the heifer's mature weight at first calving as recommended in the NASEM (2016) equations 13-26 through 13-27. The retained energy required to achieve the desired gain after pregnancy based on the target weights that we predicted was calculated using the NASEM (2016) equation 12-1. Additionally, heifer gestation requirements were calculated using the NASEM (2016) equations 13-34 through 13-44 assuming a 30 kg calf birth weight. The net energy requirements for maintenance, growth, and gestation were then combined as demonstrated by Table 20-3 of the NASEM (2016) as the total net energy for maintenance (NEm). Then, the dietary allowance for each pair was calculated based on a restriction of 66% of the total NEm requirements of each CON heifer. Heifer mineral requirements were calculated using Table 7-1 of the NASEM (2016) recommendations for gestating cows. Each week, dry matter allowances for each BW pair were adjusted based on the CON heifer's BW for maintenance requirements, growth requirements (based on NASEM (2016) calculations), and week of gestation (Table 2) that met 66% of the heifer's NEm requirements and equaled or exceeded her other nutrient requirements. Heifer BW was excluded from analyses on day 259 of gestation because of scale malfunction; however, this BW was used for diet formulation on day 259. This allowed each BW pair to receive the same dry matter allowance each day throughout the treatment period.

All heifers were removed from their individual pens 14 days before their expected calving date on March 8, 2021 to prevent any calves from being born in the pens that heifers were housed in during the treatment period. After calving, heifers and their calves were housed together in a single pasture.

## Prepartum Measurements

Beginning on day 196 of gestation, heifers were weighed and assigned a BCS (1 = emaciated; 9 = obese; Wagner et al., 1988) weekly until parturition. Heifers were weighed once at the beginning of each week at 0830 h. Heifers were allowed ad libitum access to water before being weighed; however, heifers were not fed until after they were weighed on these days. From this total BW, conceptus free live weight (CFLW) was calculated using prediction equations from Ferrell et al. (1976) to provide an estimated heifer weight without the gravid uterus. Body weights from day 259 of gestation were excluded from the BW and CFLW analyses, as the scale at the research station was not working properly. Additionally, each week heifers were scanned via ultrasonography by the same technician between the 12th and 13th ribs over the longissimus muscle for back fat thickness (BF) and ribeye area (REA), and rump fat (RF) thickness (Brethour, 1992). Dry matter intake was recorded daily. There were no refusals were observed for any of the heifers throughout the treatment period. While heifers were in the handling system, rectal temperature was recorded weekly from day 210 to day 266 of gestation to evaluate the heifer's core body temperature over time.

Blood samples were collected weekly via jugular venipuncture into a 10 mL vacutainer collection tube containing $K^2$

**Table 2.** The body weight (BW; kg) of the control heifer in each BW pair used to calculate maintenance, growth, and gestation requirements (NASEM, 2016), and the net energy (NE; Mcal/d) and crude protein (CP; g/d) provided to pair fed heifers restricted to 66% of their net energy requirements and either housed in pens filled with mud (MUD; 19.5 ± 7.9 cm) or housed in pens filled with wood chips (CON) from day 196 to day 266 of gestation.

| Day of Gestation | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 196 | 203 | 210 | 217 | 224 | 231 | 238 | 245 | 252 | 259[3] | 266 |
| **Pair 1** | | | | | | | | | | | |
| *Control BW* | 417.2 | 430.8 | 437.2 | 418.1 | 424.5 | 422.7 | 425.4 | 427.2 | 418.1 | 463.5 | 465.3 |
| *NEm, Mcal/d*[1] | 13.01 | 13.01 | 13.01 | 14.82 | 15.43 | 16.90 | 17.50 | 19.26 | 23.17 | 20.71 | --- |
| *CP, g/d*[2] | 858.21 | 858.21 | 858.21 | 977.96 | 1017.88 | 1085.68 | 1124.45 | 1204.22 | 1448.83 | 1258.89 | --- |
| **Pair 2** | | | | | | | | | | | |
| *Control BW* | 427.2 | 395.5 | 428.1 | 437.2 | 439.9 | 431.7 | 436.3 | 448.1 | 445.4 | 473.5 | 447.2 |
| *NEm, Mcal/d* | 12.71 | 14.82 | 13.61 | 13.61 | 13.92 | 15.99 | 16.29 | 15.95 | 17.76 | 13.81 | --- |
| *CP, g/d* | 838.25 | 977.96 | 898.13 | 898.13 | 918.08 | 1027.52 | 1046.91 | 997.25 | 1110.14 | 839.26 | --- |
| **Pair 3** | | | | | | | | | | | |
| *Control BW* | 431.7 | 433.6 | 434.5 | 428.1 | 428.1 | 425.4 | 425.4 | 434.5 | 438.1 | 469.8 | 439.9 |
| *NEm, Mcal/d* | 12.40 | 12.71 | 13.31 | 14.22 | 15.13 | 16.59 | 17.50 | 18.06 | 19.26 | 14.12 | --- |
| *CP, g/d* | 818.29 | 838.25 | 878.17 | 938.04 | 997.92 | 1066.29 | 1124.45 | 1128.96 | 1204.22 | 857.50 | --- |
| **Pair 4** | | | | | | | | | | | |
| *Control BW* | 432.7 | 429.0 | 429.9 | 437.2 | 435.4 | 430.8 | 438.1 | 447.2 | 452.6 | 471.7 | 449.9 |
| *NEm, Mcal/d* | 12.40 | 13.01 | 13.61 | 13.61 | 14.52 | 15.99 | 15.99 | 16.25 | 16.55 | 13.81 | --- |
| *CP, g/d* | 818.29 | 858.21 | 898.13 | 898.13 | 958.00 | 1027.52 | 1027.52 | 1016.06 | 1034.88 | 839.26 | --- |
| **Pair 5** | | | | | | | | | | | |
| *Control BW* | 435.4 | 431.7 | 435.4 | 426.3 | 420.9 | 425.4 | 427.2 | 434.5 | 423.6 | 455.3 | 448.1 |
| *NEm, Mcal/d* | 12.10 | 12.71 | 13.01 | 14.52 | 15.73 | 16.59 | 17.50 | 18.06 | 21.97 | 17.71 | --- |
| *CP, g/d* | 798.33 | 838.25 | 858.21 | 958.00 | 1037.83 | 1066.29 | 1124.45 | 1128.96 | 1373.56 | 1076.44 | --- |
| **Pair 6** | | | | | | | | | | | |
| *Control BW* | 445.4 | 446.3 | 439.0 | 441.7 | 434.5 | 434.5 | 435.4 | 449.0 | 444.4 | 444.4 | 455.3 |
| *NEm, Mcal/d* | 11.80 | 12.10 | 13.01 | 13.31 | 14.52 | 15.69 | 16.59 | 17.76 | 18.06 | 20.71 | --- |
| *CP, g/d* | 778.38 | 798.33 | 858.21 | 878.17 | 958.00 | 1008.13 | 1066.29 | 1110.14 | 1128.96 | 1258.89 | --- |
| **Pair 7** | | | | | | | | | | | |
| *Control BW* | 451.7 | 457.1 | 441.7 | 452.6 | 446.3 | 455.3 | 451.7 | 468.9 | 468.0 | 481.6 | 467.1 |
| *NEm, Mcal/d* | 11.50 | 11.50 | 12.71 | 12.40 | 13.61 | 13.58 | 14.48 | 12.94 | 13.54 | 13.81 | --- |
| *CP, g/d* | 758.42 | 758.42 | 838.25 | 818.29 | 898.13 | 872.42 | 930.58 | 809.09 | 846.72 | 839.26 | --- |

**Table 2.** Continued

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Pair 8** | | | | | | | | | | | |
| *Control BW* | 451.7 | 444.4 | 450.8 | 445.4 | 447.2 | 439.9 | 445.4 | 455.3 | 449.9 | 478.9 | 461.7 |
| *NEm, Mcal/d* | 11.50 | 12.10 | 12.10 | 13.01 | 13.31 | 15.09 | 15.39 | 15.05 | 17.15 | 13.81 | --- |
| *CP, g/d* | 758.42 | 798.33 | 798.33 | 858.21 | 878.17 | 969.36 | 988.74 | 940.80 | 1072.51 | 839.26 | --- |
| **Pair 9** | | | | | | | | | | | |
| *Control BW* | 460.8 | 459.9 | 459.0 | 456.2 | 448.1 | 450.8 | 449.9 | 459.9 | 470.7 | 475.3 | 459.9 |
| *NEm, Mcal/d* | 10.89 | 11.19 | 11.50 | 12.10 | 13.31 | 13.88 | 14.78 | 14.45 | 13.24 | 13.81 | --- |
| *CP, g/d* | 718.50 | 738.46 | 758.42 | 798.33 | 878.17 | 891.81 | 949.97 | 903.17 | 827.90 | 839.26 | --- |

[1]NEm, Mcal/d = Net energy for maintenance provided to each pair that was adjusted weekly (Mcal/d) based on the nutritional profile of the diet (Table 1).
[2]CP, g/d = Crude protein provided to each pair that was adjusted weekly (g/d) based on the nutritional profile of the diet (Table 1).
[3]Scales malfunctioned on day 259 of gestation. Therefore, although diet formulation was based on this weight, the body weights on this day have been excluded from the analyses.

EDTA. Blood samples were collected as heifers were weighed each week; therefore, blood samples were collected at approximately 0830 h and before heifers were fed that day. Blood samples were placed on ice until they were transferred back to the laboratory where they were centrifuged at 2,500 × *g* for 20 min at 4°C. Plasma collected from the K[2] EDTA tubes was transferred into microcentrifuge tubes and stored at −20°C for later quantification of plasma non-esterified fatty acid (NEFA) and cortisol concentrations. A colorimetric assay was used to determine concentration of plasma NEFA (Wako Chemicals USA, Richmond, VA) according to a protocol by Johnson and Peters (1993). Intra-assay variation for plasma NEFA was 2.82%, and inter-assay variation was 2.00%. Plasma cortisol concentration was quantified using a commercially available radioimmunoassay kit (MP Biomedicals, LLC., Solon, OH). The minimum level of detection was 1 µg/dL. All samples were run in a single assay; therefore, the intra-assay variation was 1.78%.

Beginning on day 196 of gestation, heifers were assigned a mud score at weekly intervals as follows: 1 = no tag, clean hide; 2 = small lumps of manure/mud attached to the hide in limited areas of the legs and underbelly; 3 = small and large lumps of manure/mud attached to the hide covering larger areas of the legs, side, and underbelly; 4 = small and large lumps of manure/mud attached to the hide in even larger areas along the hind quarter, stomach, and front shoulder; and 5 = lumps of manure/mud attached to the hide continuously on the underbelly and side of the animal from brisket to rear quarter (Busby and Strohbehn, 2008). Mud depth and mud temperature in each of the 9 individual mud pens was also recorded weekly. Mud depth was measured according to a procedure by Castillo et al. (2012) where a steel rod 1 meter in length with a density of 1.1 g cm$^3$ was dropped from a height of 1 meter above the ground. The rod was dropped vertically through a 2.54 cm diameter PVC pipe, and the portion of the steel rod immersed in the mud was considered the depth of the mud. Mud temperature was recorded by inserting a thermometer 75 mm into the mud in the middle of each pen. In addition to manually recording mud temperature in the experimental pens, weather data including daily solar radiation (A; Cal/cm$^2$), daily precipitation (B; mm), mean ambient air temperature (C; °C), mean soil temperature (D; °C), mean wind speed (E; km/h), and mean relative humidity (F; %) were recorded using the EARS weather station that is on the research station where this research took place (https://weather.cfaes.osu.edu/stationinfo.asp?id=3). This pen data and weather data are presented in Fig. 1 and Fig. 2A-F as descriptive results, respectively.

## Postpartum Measurements

Due to the definition of heifers, after calving the pregnant heifers became primiparous cows. Heifers were removed from their individual pens on day 266 of gestation to prevent any calves from being born in muddy conditions. Within the first 12 h after birth, cow BW and calf birth BW were recorded. Additionally, within 24 to 48 h after birth, a blood sample was obtained from each calf to quantify plasma IgG concentration. Each blood sample was collected via jugular venipuncture into a 5 mL vacutainer collection tube containing K[2] EDTA. Samples were placed on ice until they were centrifuged at 2,500 × *g* for 25 min at 4°C. Plasma from the collection tubes was frozen at −20°C for quantification of plasma IgG concentration. Calves were sampled for

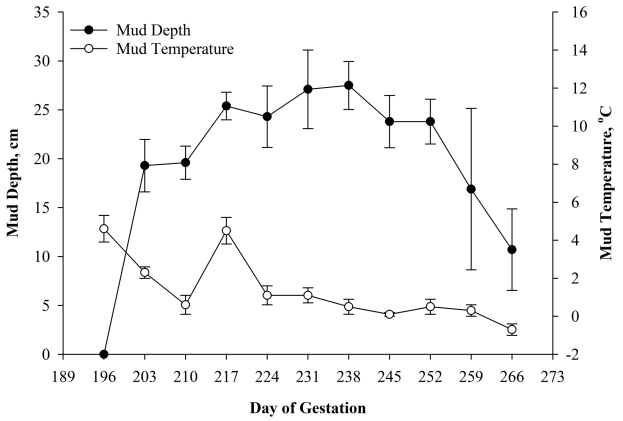


**Figure 1.** Average mud depth (cm) and mud temperature (°C) ± SD of the 9 MUD treatment pens recorded at weekly intervals from the beginning of the treatment period on day 196 of gestation until the end of the treatment period on day 266 of gestation.

blood weekly until approximately 28 days of age to quantify plasma IgG concentrations over the first 28 days of life. A sandwich enzyme-linked immunosorbent assay was used to quantify plasma IgG concentration (Bethyl Laboratories, Inc., Montgomery, TX). The inter-assay and intra-assay coefficients of variation were 3.9% and 0.8%, respectively. Both heifer and calf BW were continually recorded every week until weaning when calves were 196.7 ± 3.6 days of age.

### Statistical Analyses

Heifer was considered the experimental unit with 9 replications per treatment. Body weight pair was considered the blocking criteria. Heifer BW, BCS, BF, REA, RF, plasma NEFA concentration, plasma cortisol concentration, rectal temperature, mud score, and calf plasma IgG concentration were analyzed using the MIXED procedure of SAS (9.4, SAS Inst. Inc., Cary, NC). The model included treatment, day of gestation, and their interaction as fixed effects. The model also included block and heifer within block by treatment as the random effects. A covariance structure was used to account for the error's correlation due to the repeated measures over time. The covariance structure that resulted in the lowest AIC for each repeated measures variable was selected. For BW, BF, and RF the first-order autoregressive structure with heterogenous variances was used, and for BCS, REA, plasma NEFA concentration, plasma cortisol concentration, rectal temperature, mud score, and calf plasma IgG concentration the autoregressive covariance structure was used. A simple linear regression was performed for each treatment using day of gestation as the independent variable and CFLW as the dependent variable to evaluate the slope of CFLW change for each treatment throughout the experimental period. Heifer BW at parturition, gestation length, calf birth weight, calf weaning weight, and heifer BW at weaning were analyzed using the MIXED procedure of SAS. Similar to the previous models, the model included block and either heifer or calf within block by treatment as the random effects depending on if it was a heifer or calf variable. The assumptions of normality and homogeneity of variance were evaluated using the residuals plots in SAS for all variables. No variables violated these assumptions, therefore, there was no need for transformations. Differences were considered significant if $P \leq 0.05$ and marginally significant if $0.05 < P \leq 0.10$. If the day of gestation × treatment interaction was significant, the PDIFF option of SAS was used for mean separation. Data are presented as LS means ± SEM.

## RESULTS

### Mud Measurements, Weather Observations, and Mud Scores

Mud depth and mud temperature of the experimental pens are presented in Fig. 1 as descriptive data. The mud in the pens was artificially created and was targeted to be 20 to 30 cm in depth. At the beginning of the treatment period on day 196 of gestation, the pens were not yet muddy and therefore mud depth could not be measured. Throughout the treatment period, the manually recorded temperatures of the MUD pens were consistently less than the mean soil temperature recorded by the EARS weather station. Though the manually recorded temperatures were less than the weather station's soil temperature estimates, they did follow the same pattern and were reflective of the mean soil temperature.

Weather data obtained from the EARS weather station is presented in Fig. 2A-F as descriptive statistics. The treatment period began on December 14, 2020 which was day 196 of gestation and ended on February 22, 2021 at day 266 of gestation. Data were obtained from the research weather station to determine how many days of the treatment period heifers were exposed to precipitation. Of the 70-day treatment period, heifers experienced precipitation (rain or snow) for 34 days (49% of the treatment period). This accumulated to a total of approximately 144.8 mm of precipitation throughout the 70-day treatment period.

There was a treatment × day of gestation effect ($P < 0.01$) for mud score (Fig. 3). By design, all heifers started the study at a similar mud score ($P = 1.0$) as they were group fed and housed in the EARS feedlot facility for two weeks before the start of the treatment period during the dietary adaptation period. At the start of the treatment period, both treatments started at a mud score of 2 (small lumps of manure/mud attached to the hide in limited areas of the legs and underbelly). By the end of the treatment period, heifers in the CON treatment had a mud score of approximately 1.7, compared with the MUD treatment with a mud score of approximately 3.3 (small and large lumps of manure/mud attached to the hide covering larger areas of the legs, side, and underbelly).

### Heifer Prepartum Measurements

By experimental design, heifers in each BW pair were fed the same amount of dry matter each day and there were no refusals from any of the heifers throughout the treatment period. There was a treatment × day of gestation effect ($P < 0.01$) for BW (Fig. 4) and BCS (Fig. 5). At the start of the study on day 196 of gestation, both treatments were of similar BW ($P = 0.99$) and BCS ($P = 0.65$). Though each treatment started at similar BW and heifers were pair fed throughout the treatment period, heifers in the MUD treatment decreased BW throughout the study and weighed 43.5 kg less than the heifers in the CON treatment on day 266 of gestation, while heifers in the CON treatment increased their BW throughout the study ($P < 0.01$). Heifers in the CON and MUD treatment started similar BCS (6.3 ± 0.2 and 6.4 ± 0.2, respectively) on day 196 of gestation; however, by day 266 of gestation

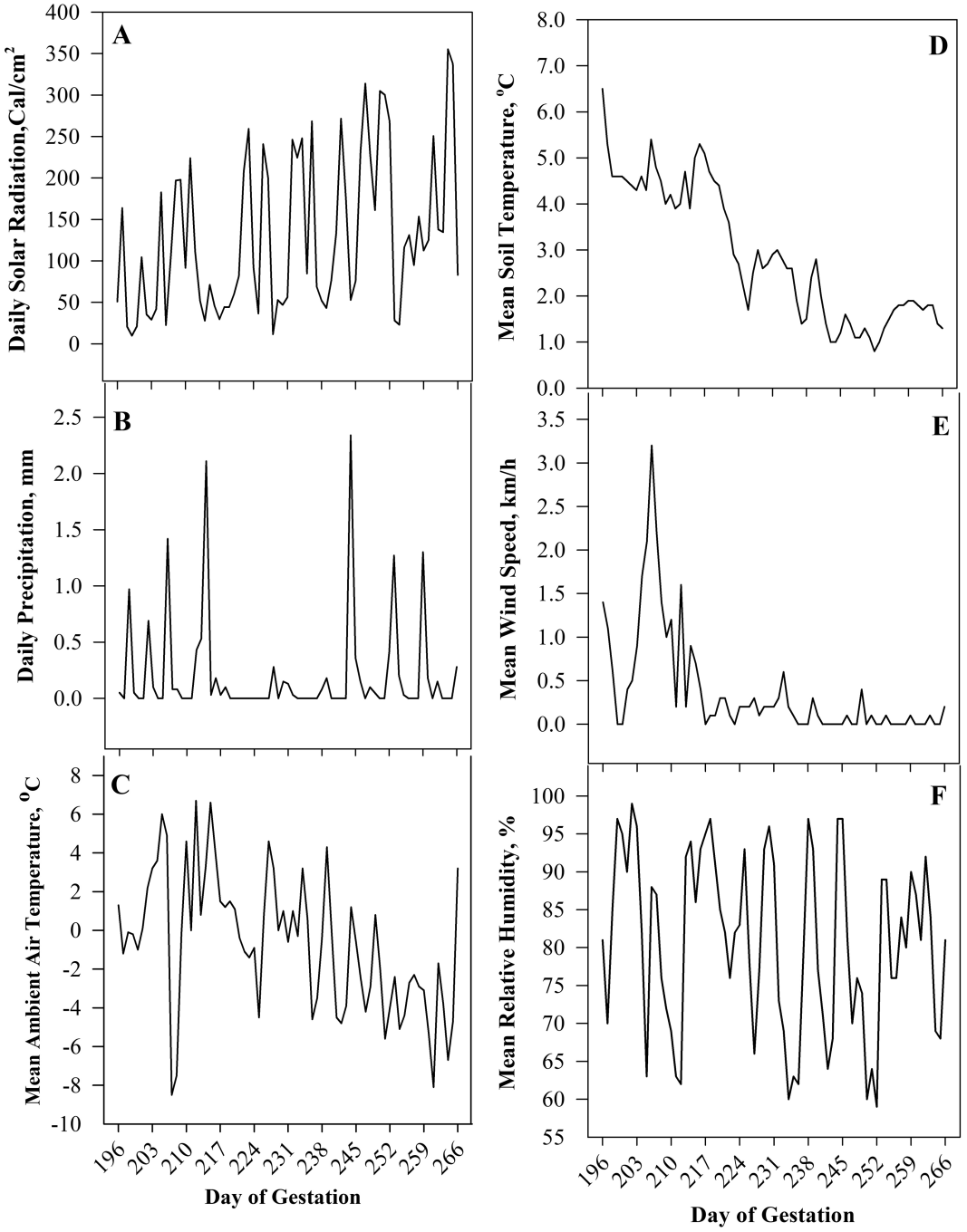


**Figure 2.** Daily solar radiation (A; Cal/cm²), daily precipitation (B; mm), mean ambient air temperature (C; °C), mean soil temperature (D; °C), mean wind speed (E; km/h), and mean relative humidity (F; %) from the start of the treatment period on day 196 of gestation (December 14, 2020) to the end of the treatment period on day 266 of gestation (February 22, 2021) at the Eastern Agricultural Research Station Caldwell, Ohio.

the heifers in the MUD treatment decreased 2.1 condition score points and were a BCS of 4.3, compared with the CON heifers that maintained a BCS of 6.1 ($P < 0.01$). There was evidence for a treatment × day of gestation effect ($P < 0.01$) for CFLW (Fig. 6). Heifers in both treatments started at similar CFLW on day 196 of gestation ($P = 1.00$); however, by day 266 of gestation the heifers in the MUD treatment had a CFLW that was 43.5 kg less than the CON treatment ($P < 0.01$). Using simple linear regression to evaluate the slope of CFLW over the treatment period, the heifers in the CON treatment decreased their CFLW by 2.1 kg per week, and the MUD heifers decreased their CFLW by approximately 6.8 kg per week. Both the slope of –2.1 kg/week for the CON treatment and the slope of –6.8 kg/week for the MUD treatment were significantly different from zero ($P < 0.01$).

There was a treatment × day of gestation effect ($P = 0.02$) for BF thickness (Fig. 7). Heifers in the CON and MUD treatments started at similar BF thicknesses of 0.6 and 0.7 cm, respectively ($P = 0.37$). However, by day 266 of gestation heifers in the CON treatment maintained their BF thickness of approximately 0.6 cm compared with the MUD treatment that only had a BF thickness of approximately 0.4 cm ($P < 0.01$). There was no evidence for a treatment × day of gestation effect ($P = 0.11$) or a treatment effect ($P = 0.22$) for

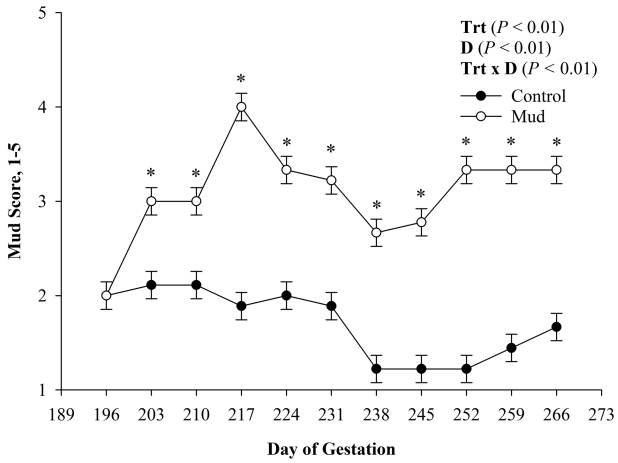


**Figure 3.** Mean prepartum heifer mud score ± SEM measured weekly from day 196 of gestation (start of treatment period) to day 266 of gestation (end of treatment period) of pair fed heifers restricted to 66% of their net energy requirements and either housed in 19.5 ± 7.9 cm of mud (Mud) or wood chips (Control) presented with Treatment (Trt), Day of Gestation (D), and Treatment × Day of Gestation (Trt × D) effects and $P \leq 0.05$ represented with *.

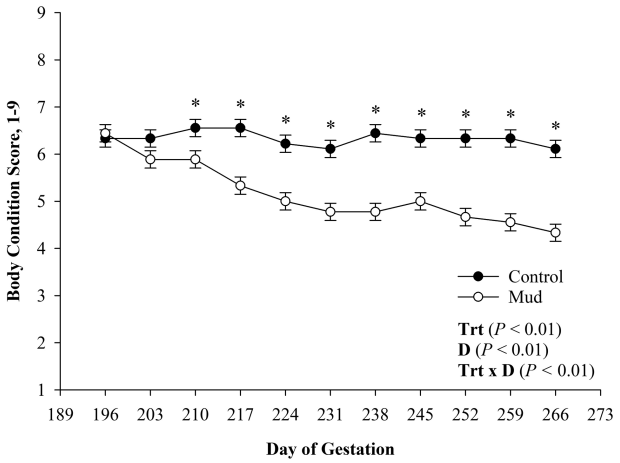


**Figure 5.** Mean prepartum heifer body condition score ± SEM measured weekly from day 196 of gestation (start of treatment period) to day 266 of gestation (end of treatment period) of pair fed heifers restricted to 66% of their net energy requirements and either housed in 19.5 ± 7.9 cm of mud (Mud) or wood chips (Control) presented with Treatment (Trt), Day of Gestation (D), and Treatment × Day of Gestation (Trt × D) effects and $P \leq 0.05$ represented with *.

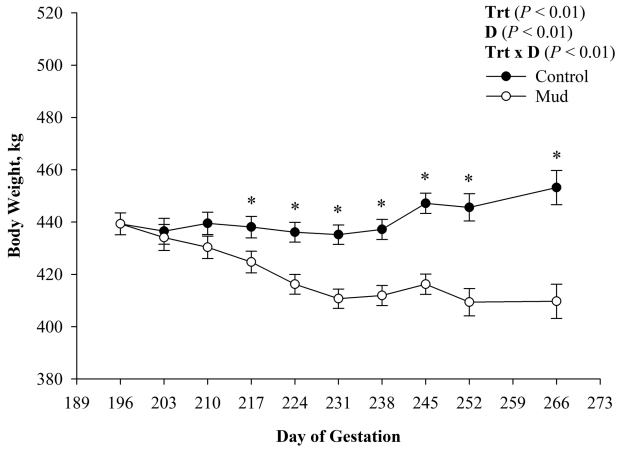


**Figure 4.** Mean prepartum heifer body weight ± SEM measured weekly from day 196 of gestation (start of treatment period) to day 266 of gestation (end of treatment period) of pair fed heifers restricted to 66% of their net energy requirements and either housed in 19.5 ± 7.9 cm of mud (Mud) or wood chips (Control) presented with Treatment (Trt), Day of Gestation (D), and Treatment × Day of Gestation (Trt × D) effects and $P \leq 0.05$ represented with *.

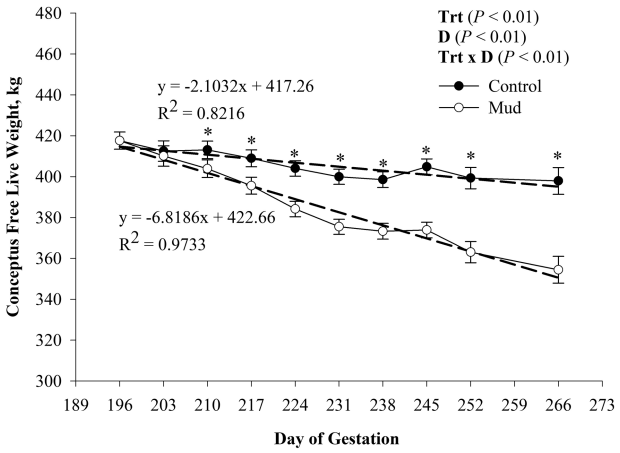


**Figure 6.** Mean prepartum conceptus free live weight (CFLW) ± SEM estimated using the weekly heifer body weight from day 196 of gestation (start of treatment period) to day 266 of gestation (end of treatment period) of pair fed heifers restricted to 66% of their net energy requirements and either housed in 19.5 ± 7.9 cm of mud (Mud) or wood chips (Control) presented with Treatment (Trt), Day of Gestation (D), and Treatment × Day of Gestation (Trt × D) effects and $P \leq 0.05$ represented with *. Conceptus free live weight was estimated using equations by Ferrell et al. (1976).

REA (Fig. 8); however, there was a day of gestation effect ($P < 0.01$). Both treatment groups decreased their REA toward the end of the treatment period. Similar to BF, there was a treatment × day of gestation effect for RF (Fig. 9; $P < 0.01$). Heifers in both treatments started at similar RF ($P = 0.71$). While the CON treatment was able to maintain their RF, the MUD treatment continually decreased their RF throughout the treatment period. By the end of the treatment period on day 266 of gestation, the MUD treatment had a rump fat thickness of approximately 0.3 cm less compared with the CON treatment ($P < 0.01$).

## Heifer Prepartum Metabolites

There was no evidence for a treatment × day of gestation effect ($P = 0.98$) or a treatment effect ($P = 0.62$) for NEFA concentration (Fig. 10); however, there was a day of gestation effect ($P < 0.01$). All heifers had their greatest NEFA concentration on day 224 of gestation. Both treatment groups started the 14-day dietary adjustment period at similar plasma NEFA concentration on day 182 ($P = 0.70$) and day 189 of gestation ($P = 0.81$). After the peak in plasma NEFA concentration on day 224 of gestation, both treatments decreased their plasma NEFA concentration by day 266 of gestation.

Similar to plasma NEFA concentration, there was no evidence for a treatment × day ($P = 0.80$) or a treatment ($P = 0.59$) effect for plasma cortisol (Fig. 11); however, there was a day of gestation effect ($P < 0.01$). Both treatment groups decreased their plasma cortisol concentration within the first

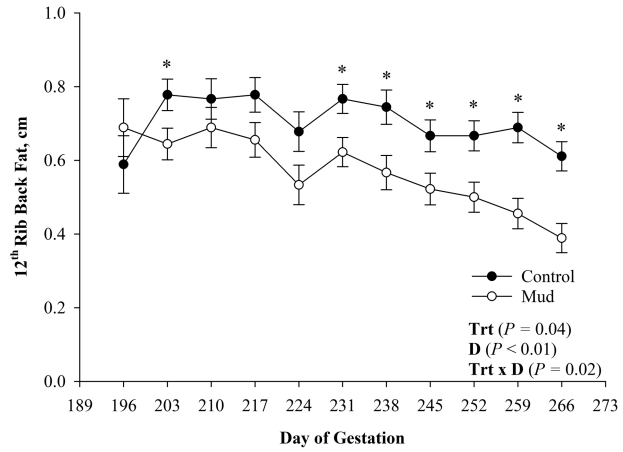


**Figure 7.** Mean prepartum heifer 12th rib back fat thickness ± SEM measured weekly from day 196 of gestation (start of treatment period) to day 266 of gestation (end of treatment period) of pair fed heifers restricted to 66% of their net energy requirements and either housed in 19.5 ± 7.9 cm of mud (Mud) or wood chips (Control) presented with Treatment (Trt), Day of Gestation (D), and Treatment × Day of Gestation (Trt × D) effects and $P \leq 0.05$ represented with *.

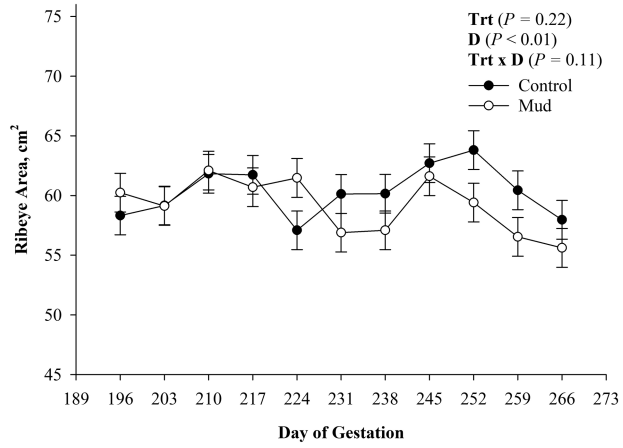


**Figure 8.** Mean prepartum heifer ribeye area ± SEM measured weekly from day 196 of gestation (start of treatment period) to day 266 of gestation (end of treatment period) of pair fed heifers restricted to 66% of their net energy requirements and either housed in 19.5 ± 7.9 centimeters of mud (Mud) or wood chips (Control) presented with Treatment (Trt), Day of Gestation (D), and Treatment × Day of Gestation (Trt × D) effects.

week of the treatment period, had an increase on day 224 of gestation, and then subsequently decreased their plasma cortisol concentration until the end of the treatment period.

There was no evidence of a treatment × day of gestation ($P = 0.72$) or a treatment ($P = 0.58$) effect for rectal temperature (Fig. 12). There was a day of gestation effect ($P < 0.01$) such that all heifers had their peak rectal temperature on day 238 of gestation, and then decreased their rectal temperatures in the subsequent week.

### Parturition and Postpartum Measurements

After birth, heifers in the MUD treatment weighed 30.3 kg less than the CON treatment (Table 3; $P < 0.01$). While heifer BW was different, calf birth weight was not different between the treatments (Table 3; $P = 0.34$). There was, however, evidence

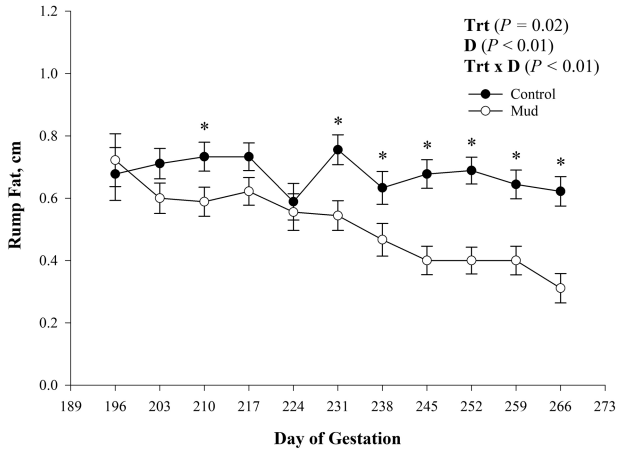


**Figure 9.** Mean prepartum heifer rump fat thickness ± SEM measured weekly from day 196 of gestation (start of treatment period) to day 266 of gestation (end of treatment period) of pair fed heifers restricted to 66% of their net energy requirements and either housed in 19.5 ± 7.9 cm of mud (Mud) or wood chips (Control) presented with Treatment (Trt), Day of Gestation (D), and Treatment × Day of Gestation (Trt × D) effects and $P \leq 0.05$ represented with *.

for a marginally significant difference (Table 3; $P = 0.06$) in gestation length such that heifers in the MUD treatment calved approximately 3.1 days earlier than heifers in the CON treatment. By weaning, there was no evidence for a difference in heifer BW between the MUD and CON treatments (Table 3; $P = 0.44$). There was similarly no evidence for a difference in calf weaning weight (Table 3; $P = 0.63$).

There was no evidence for a treatment ($P = 0.63$) or a treatment × day effect ($P = 0.37$) for plasma IgG concentration (Fig. 13) in the calves. There was a day effect ($P < 0.01$), such that all calves had their greatest plasma IgG concentration within 24 to 48 h after birth.

## DISCUSSION

We accept our hypothesis that energy restriction and muddy conditions increase net energy requirements and cause a decrease in BW and BCS in pregnant heifers during late gestation that is greater than that of heifers that are energy restricted and housed on wood chip bedding. Based on the present results, muddy environmental conditions caused heifers to weigh approximately 43.5 kg less than the CON treated heifers by day 266 of gestation, though they were pair fed throughout the treatment period to the CON heifers' BW that were housed in pens filled with sawdust and wood chips. While we observed this decrease in BW for the heifers in the MUD treatment, there was no evidence of a treatment effect on calf birth weight. This lack of evidence for a difference in calf birth weight allowed us to assume that fetal growth was similar between treatments, and we estimated CFLW using equations proposed by Ferrell et al. (1976) as an indicator of heifer BW without the gravid uterus. Since pregnant heifers are expected to continue to grow throughout gestation to reach the NASEM (2016) recommended target of 80% of expected mature weight by first calving, we expected CFLW to increase as heifers progress throughout gestation and approach their target BW for calving. This increase in CFLW is only possible; however, if a heifer's maintenance, growth, and gestation requirements are met.

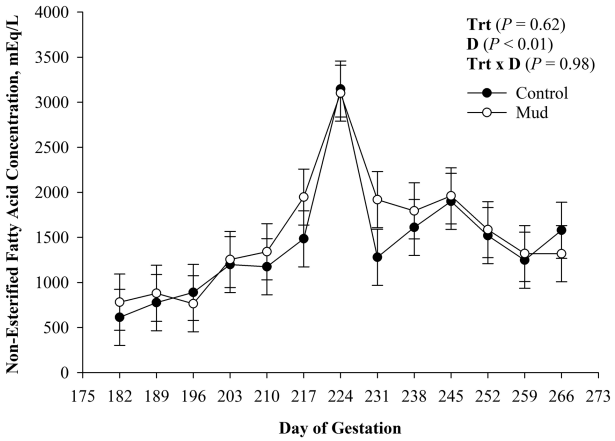


**Figure 10.** Mean prepartum heifer plasma non-esterified fatty acid concentration ± SEM measured weekly from day 196 of gestation (start of treatment period) to day 266 of gestation (end of treatment period) of pair fed heifers restricted to 66% of their net energy requirements and either housed in 19.5 ± 7.9 cm of mud (Mud) or wood chips (Control) presented with Treatment (Trt), Day of Gestation (D), and Treatment × Day of Gestation (Trt × D) effects.

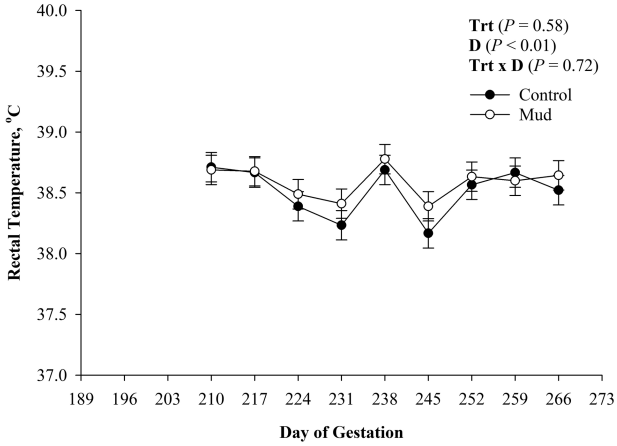


**Figure 12.** Mean prepartum rectal temperature ± SEM measured weekly from day 210 of gestation to day 266 of gestation (end of treatment period) of pair fed heifers restricted to 66% of their net energy requirements and either housed in 19.5 ± 7.9 cm of mud (Mud) or wood chips (Control) presented with Treatment (Trt), Day of Gestation (D), and Treatment × Day of Gestation (Trt × D) effects.

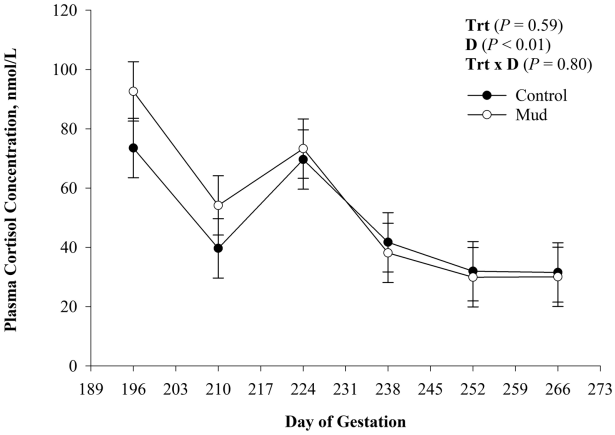


**Figure 11.** Mean prepartum heifer plasma cortisol concentration ± SEM measured weekly from day 196 of gestation (start of treatment period) to day 266 of gestation (end of treatment period) of pair fed heifers restricted to 66% of their net energy requirements and either housed in 19.5 ± 7.9 cm of mud (Mud) or wood chips (Control) presented with Treatment (Trt), Day of Gestation (D), and Treatment × Day of Gestation (Trt × D) effects.

Heifers in the MUD treatment decreased their CFLW by 63.3 kg, while heifers in the CON treatment decreased their CFLW by 19.7 kg. These results indicate that neither treatment group was meeting their maintenance, growth, and gestation requirements. The reduction in CFLW for the CON and MUD treatments was expected since each pair was only provided 66% of the CON heifer's net energy requirements for maintenance, growth, and gestation. Nevertheless, although heifers in the CON treatment decreased their CFLW, they increased their total BW indicating that they were able to provide for adequate fetal growth. Furthermore, heifers in both treatments were able to produce calves that were greater than the 30 kg target birth weight and were able to maintain fetal growth while simultaneously decreasing their own CFLW. This is a noteworthy result, as the NASEM (2016) indicates that the prioritization of nutrient is (1) basal metabolism, (2) activity to gather food, (3) growth, (4) basic energy reserves, (5) maintenance of pregnancy, (6) lactation to support an existing offspring, (7) accumulation of additional energy reserves, (8) estrous cycles and initiation of pregnancy, and (9) accumulation of excess energy reserves. However, the present results challenge the NASEM (2016), and indicate that heifers in the CON treatment that were energy restricted and heifers in the MUD treatment that were both energy restricted and housed in muddy conditions during late gestation that further increased their energy requirements prioritized fetal growth above their own maintenance and/or growth. Additionally, these results warrant further discussion as to the NASEM (2016) and its recommendations for the net energy calculations for a pregnant heifer. In this experiment, individual net energy requirements were calculated for heifer maintenance, growth, and gestation. Table 20-3 of the NASEM (2016) indicates that it is acceptable to then add these net energy requirements to get a total net energy, and that this total net energy required is referred to as NEm. Table 20-3 then indicates that the diet should be formulated based on NEm, and that the net energy for gain (NEg) of each feed ingredient should not be used in diet formulation. When calculating energy requirements in this manner, it is assumed that maintenance, growth, and gestation all have similar efficiencies of use, as everything is put on a NEm basis, rather than separately using NEm and NEg. However, the "Reproduction" chapter of the NASEM (2016) contradicts Table 20-3, and states that average daily gain and net energy requirements for gain (NEg) should be used to calculate the requirements needed to achieve the target weights for the pregnant heifers rather than just NEm. Based on the present results and the utilization of Table 20-3 in this experiment, it seems that Table 20-3 is accurate in assuming that a heifer is able to use the net energy available for maintenance for either fetal growth or their own growth at a similar efficiency.

As was expected, the linear regression for CFLW indicates that neither treatment group was meeting their net energy requirements, as each treatment's slope was significantly different from zero. Using Table 13-4 of the NASEM (2016) and

**Table 3.** LS Mean ± SEM heifer body weight (BW; kg) at parturition, gestation length (days; d), calf birth weight (kg), calf weaning weight (kg), and heifer BW (kg) at weaning for pair fed heifers restricted to 66% of their net energy requirements and either housed in pens filled with mud (MUD; 19.5 ± 7.9 cm) or housed in pens filled with wood chips (CON) from day 196 to day 266 of gestation.

| | Control | Mud | P-value |
|---|---|---|---|
| Heifer BW at parturition, kg | 413.3 ± 6.2 | 383.0 ± 6.2 | <0.01 |
| Gestation length, d | 277.9 ± 1.1 | 274.8 ± 1.1 | 0.06 |
| Calf birth weight, kg | 32.9 ± 1.7 | 30.9 ± 1.7 | 0.34 |
| Calf weaning weight, kg | 238.5 ± 7.2 | 233.8 ± 7.7 | 0.63 |
| Heifer BW at weaning, kg | 480.6 ± 9.4 | 472.9 ± 9.4 | 0.44 |

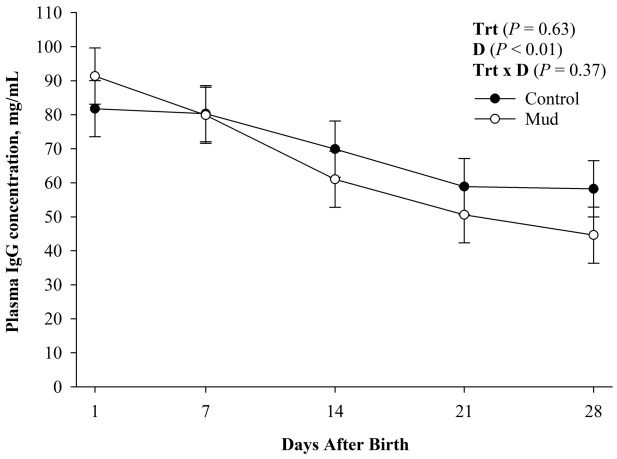


**Figure 13.** Mean prepartum calf plasma IgG concentration ± SEM measured weekly from 24 to 48 h after birth until 28 days of age of calves born to pair fed heifers restricted to 66% of their net energy requirements and either housed in 19.5 ± 7.9 cm of mud (Mud) or wood chips (Control) presented with Treatment (Trt), Day of Gestation (D), and Treatment × Day of Gestation (Trt × D) effects.

the BCS of the heifers in the CON treatment at the start of this study (BCS = 6.3 ± 0.2), it is estimated that 1 kg of empty body weight loss is equivalent to 6.38 Mcal of energy. The decrease in estimated CFLW for the CON heifers of 2.1 kg each week suggests an energetic cost of 13.4 Mcal/week. This 13.4 Mcal/week multiplied by the 10-week treatment period suggests a total energetic cost of 134 Mcal. The average energetic cost on a per day basis for the CON heifers in this study can be estimated by dividing the 134 Mcal by the 70-day treatment period. This equation results in an estimated 1.9 Mcal/day of additional energy required by the CON heifers to meet their net energy requirements under our experimental conditions. We speculate that the decrease in CFLW that we observed in the CON treatment could be because heifers were periodically cold stressed, as the average ambient temperature during the treatment period was −0.6°C and we did not incorporate cold stress into our weekly calculations for the heifer's nutrient requirements and therefore the dry matter allowances for both treatments. Again, using Table 13-4 of the NASEM (2016) and the BCS of the heifers in the MUD treatment at the start of this study (BCS = 6.4 ± 0.2), it is estimated that 1 kg of empty body weight loss is equivalent to 6.38 Mcal of energy. Performing the same calculations for the heifers in the MUD treatment, the decrease in CFLW of 6.8 kg for the MUD heifers each week suggests an energetic cost of 43.4 Mcal/week. This 43.4 Mcal/week multiplied by the 10-week treatment period suggests a total energetic cost of 433.8 Mcal. The average energetic cost of mud on a per day basis for the heifers in the MUD treatment can be estimated by dividing the 433.8 Mcal by the 70-day treatment period. This equation results in an estimated 6.2 Mcal/day of additional energy that was required by the MUD heifers to meet their net energy requirements under our experimental conditions. Since our results indicate that the CON heifers were not meeting their net energy requirements, and the heifers in the MUD treatment were being pair fed to the CON heifers, we feel that the energetic cost of mud should be considered as the difference between the additional 6.2 Mcal/day of energy required by the MUD heifers and the additional 1.9 Mcal/day of energy required by the CON heifers. This difference is equivalent to a 4.3 Mcal/day increase in net energy requirements that can be attributed to the MUD treatment under our experimental conditions. The estimated extra energy to maintain CFLW in heifers is similar to the estimated net energy required by mature cows in mud (3.9 Mcal/day; Nickles et al. 2022). Although we do not have data on behavior, our observation from both studies is that cows and pregnant heifers placed in mud stand for a greater amount of time compared with the control cows placed on wood chips. We speculate the increase in standing behavior for the mud-treated animals indicates a change in energy demand and discomfort. Similarly, the NRC (1981) suggests the greatest depression in feed intake of feedlot cattle occurs because of mud especially when access to feed is limited and when there is a lack of suitable bedded area for the animals. We, therefore, suggest that a bedded area with no mud will reduce the extra energy requirement in gestating cows and this may be a least cost option in managing beef cows in a muddy environment. The effects of a suitable bedded area on gestating cow net energy requirements; however, is yet to be investigated.

To the best of our knowledge, the NASEM (2016) only incorporates mud into model equations for net energy in equation 11-7 to calculate external insulation in the "Maintenance Considerations" chapter. In this equation, the reader has the option to use a value of 1 (no mud), 0.8 (some mud on lower body), or 0.2 (heavily covered with mud) when calculating the external insulation value. While the NASEM (2016) acknowledges that external insulation is related to hair depth and is affected by wind, precipitation, mud, and hide thickness, we propose that the energetic cost of mud for a pregnant heifer is much greater than that predicted by the equations in the cold stress section of the NASEM (2016). Using calculations 11-3 through 11-13, we calculated the additional net energy that the NASEM (2016) predicts for cattle that are heavily covered with mud (MUD = 0.2). We also made the assumptions based on the average weather data during the

treatment period of this study that wind speed was 0.4 km/h and effective ambient temperature was −0.6°C. Additionally, we assumed that hair depth of our heifers was 7.5 cm, the hide thickness of our heifers was average (HIDE = 1), and that tissue insulation was equal to 9 (average of recommended 6.0–12.0 for adult cattle). Using these assumptions, we modeled the additional net energy required for day 266 of gestation (end of the treatment period) and for the estimated 80% of mature body weight which would be 435 kg. We chose to model the 80% of mature weight, as heifers in this study had already achieved this target BW at the start of the study, and if provided sufficient energy should have been able to maintain this weight throughout the treatment period. This equated to an additional 1.6 Mcal of net energy required for heifers that are heavily covered in mud and exposed to the previously mentioned climatic conditions. This calculated value from the NASEM (2016) is approximately 2.7 Mcal less than the 4.3 Mcal/day that we have estimated the energetic cost of mud to be under our experimental conditions.

When a ruminant enters negative energy balance, body tissues are typically mobilized in the reverse order in which they were deposited (Chilliard et al., 1998). In cattle, tissue mobilization begins with adipose tissue, followed by muscle, and lastly bone (Chilliard et al., 1998). Additionally, adipose tissue has a hierarchical order in which it will be mobilized, starting with subcutaneous fat, followed by perirenal fat, omental/mesenteric, intermuscular, and finally intramuscular fat (Chilliard et al., 1998). We hypothesized that heifers in the MUD treatment would have increased net energy requirements and would mobilize their body fat stores to help meet those requirements. We accept our hypothesis, as the decrease in BW and body condition by heifers in the MUD treatment is supported by the reduction in both 12th rib BF and RF. While heifers mobilized their adipose stores, we did not observe a reduction in ribeye area. Heifers in both treatments started with a similar BF on day 196 of gestation; however, heifers in the CON treatment had a BF thickness of 0.69 cm on day 266 of gestation compared with heifers in the MUD treatment that had a BF thickness of 0.39 cm. This is also reflected in the RF thickness, where heifers started on day 196 of gestation with similar RF thickness. By day 266 of gestation, heifers in the CON treatment had a RF thickness of 0.62 cm compared with heifers in the MUD treatment that had a RF thickness of only 0.31 cm. The present results indicate that as heifers in the MUD treatment were not meeting their nutritional requirements, they were mobilizing adipose stores in an attempt to meet their energetic demands. However, plasma NEFA concentration was not increased in the MUD treatment compared with the heifers in the CON treatment as we expected. It has previously been demonstrated that plasma NEFA concentration will increase in response to fasting or feed restriction as fat is mobilized to maintain energy homeostasis in the body (DiMarco et al., 1981). Although the heifers in the MUD treatment did mobilize their back and rump fat stores and were not meeting their net energy requirements, they were not in a fasted or feed restricted state which could be why we did not observe an accompanied response in plasma NEFA concentration. It is also possible that as heifers in both treatments were nutrient restricted and were mobilizing body fat stores, they were using these NEFA's for an energy substrate and is why there was no difference in plasma NEFA concentration between the two treatments.

Though the heifers in the MUD treatment decreased their BW, CFLW, and BCS during late gestation, neither calf birth weight nor weaning weight were affected. Our results contradict those of several other authors that have demonstrated a decrease in calf birth weight in response to maternal nutrient restriction in pregnant beef heifers (Corah et al., 1975; Spitzer et al., 1995; Cafe et al., 2006). In the study of Spitzer et al. (1995), pregnant heifers were either managed to calve with a BCS of 5 to 6 or managed to calve with a BCS of 4 to 5 during the last 90 days of gestation. The authors found that calf birth weight was significantly increased as heifers increased with each BCS unit; however, the authors did not mention the pattern of change in heifer BW during the last 90 days of gestation to obtain the different condition score of the heifers. The pattern of change is important because previous nutrition and nutrient stores of the dam can influence fetal nutrient supply (Barker and Clark, 1997). In addition, although Spitzer et al. (1995) reported differences in birth weight, weaning weight was not influenced by heifer BCS during late gestation, which is similar to the present results and indicative of the influence of post-partum nutrition. Corah et al. (1975) demonstrated a similar effect of heifer prepartum nutrition on calf birth weight. Heifers in the Corah et al. (1975) study were assigned to either 100% (17.6 Mcal of Digestible Energy/day) or 65% (11.4 Mcal of Digestible Energy/day) of the NRC (1970) recommended energy requirements during the last 100 days of gestation. The authors found the heifers fed 65% of NRC (1970) recommended energy requirements decreased their BW by only 5.8 kg, and that calf birth weight was approximately 2 kg less and weaning weight was approximately 13 kg less compared with calves born to heifers in the 100% of NRC (1970) recommended energy requirements treatment. The BW loss recorded by Corah et al. (1975) is less than what we observed in the present study; therefore, it is surprising that the authors observed a significant effect on calf birth weight. Cafe et al. (2006) also reported a difference in calf birth weight and weaning weight when heifers were managed on either a high or low plane of nutrition from conception to calving. Heifers that were in the low plane of nutrition treatment had calves that weighed approximately 3.6 kg less than calves born to heifers in the high plane of nutrition treatment. It is difficult, however, to compare the results of Cafe et al. (2006) to our study because of the lack of dry matter intake and nutrient intake data provided by Cafe et al. (2006). While our results do not agree with these three studies, they do align with Long et al. (2021) assigned heifers to receive either 100% or 70% of the NRC (2000) energy requirements from day 158 to day 265 of gestation at which heifers were slaughtered. The authors reported no differences in fetal weight, the weight of the gravid uterus, the weight of the empty uterus, number of placentomes, or placentome weight although the heifers in the restricted treatment decreased their BW by approximately 30 kg. In a previous study performed by Nickles et al. (2022) evaluating the effects of muddy conditions on mature cows, the dams in the MUD treatment decreased their BW by 37.4 kg with no effect on calf birth weight. The decrease in BW we observed in the MUD treatment is greater than that reported by any of these studies; however, the heifers were in a good to fleshy body condition at the start of the treatment period. At the start of the treatment period, heifers in the CON and MUD treatments were a BCS of approximately 6.3 ± 0.2 and 6.4 ± 0.2, respectively. While Spitzer et al. (1995) managed some heifers

at a BCS of 5 to 6 during the last 90 days of gestation, the heifers in the present study were still at a greater BCS. We cannot compare heifer BCS in the present study to Corah et al. (1975) and Cafe et al. (2006), as these authors only reported live weight change and not BCS. However, it is likely that the heifers in the present study were still at a greater BCS than the heifers in those studies. It is possible that the heifers used in this study were in such good condition on day 196 of gestation that they were able to decrease their own BW to support fetal growth as they had sufficient body fat stores to mobilize and use as an energy substrate during the period of late gestation nutrient restriction. Additionally, heifers in the MUD treatment had increased their BW by weaning and there was no evidence of a BW difference between treatments at weaning. This indicates that heifers in the MUD treatment were able to adequately increase their BW after calving and could explain why we did not observe a difference in calf weaning weight. It is also possible that the present study is underpowered to detect a difference in calf birth weight, and these results should be interpreted with that in consideration.

There was evidence for a marginally significant difference in gestation length. Heifers in the MUD treatment calved nearly 3 days earlier than heifers in the CON treatment. Although Nickles et al. (2022) evaluated the effects of muddy conditions on mature cows during late gestation, the authors also reported a numerically shorter gestation length for cows housed in muddy conditions. As a longer gestation period allows for additional fetal growth, gestation length and calf birth weight are positively correlated (Holland and Odde, 1992). It has also previously been reported that fetal plasma cortisol concentration is central to the parturition process, and that maternal plasma cortisol increases significantly and peaks around 2 days before parturition (Patel et al., 1996). In the same study of Patel et al. (1996), a single cow that gave birth prematurely had 100% greater plasma cortisol concentration on the day of parturition than cows giving birth at term. In humans, it has also been demonstrated that women in late pregnancy exposed chronic stress, classified as one or more stressful life event, had greater salivary cortisol concentrations compared with women without chronic stress (Obel et al., 2005). It is possible that we did not detect any differences for plasma cortisol concentration between the two treatment groups because of the frequency of once a week blood sampling in the current study. Based on the results, we reject our hypothesis that heifers housed in muddy conditions would be placed under chronic stress and would therefore have a greater plasma cortisol concentration and shorter gestation lengths compared with heifers housed in pens with bedding. We observed a similar pattern for plasma cortisol in both treatments, such that plasma cortisol decreased as gestation progressed, and heifers had their least plasma cortisol concentration on day 266 of gestation. It is possible that we did not observe the same increase in plasma cortisol towards the end of gestation as suggested by Patel et al. (1996) as the last blood sample was taken on day 266 of gestation and the average calving date for the MUD and CON treatments were 8.8 and 11.9 days later, respectively.

In addition to not observing evidence for a difference in calf birth weight or weaning weight, calf plasma IgG concentration within the first 28 days after birth was similar between the two dam treatments. Adequate colostrum ingestion in the first 24 h after calving is crucial to newborn calves as they are born with little or no immunoglobulins because of the lack of transplacental transfer from maternal to fetal circulation during pregnancy (Michanek et al., 1989). Cows begin colostrogenesis during late pregnancy when the mammary cells are proliferating and differentiating to prepare for the subsequent lactation (Baumrucker et al., 2010). Therefore, we hypothesized that as heifers in the MUD treatment would have greater net energy requirements, they would not be able to devote adequate energy to colostrogenesis and their calves would have decreased plasma IgG concentration after birth. Wittum and Perino (1995) classified serum IgG concentrations of >1600 mg/dL as adequate passive transfer, 800 to 1600 mg/dL as marginal passive transfer, and <800 mg/dL as failure of passive transfer. All calves in this study were classified as having adequate passive transfer, causing us to reject our hypothesis that heifers in the MUD treatment would have calves with a reduced plasma IgG concentration after birth.

In conclusion, heifers that were only provided 66% of their total net energy requirement and exposed to muddy environmental conditions decreased their total maternal BW while heifers that were only provided 66% of their total net energy requirement but not exposed to muddy environmental conditions increased their total maternal BW as gestation progressed. Though both treatments decreased their CFLW, the heifers in the MUD treatment decreased their CFLW by 6.8 kg/week compared with only 2.1 kg/week for heifers in the CON treatment. This decrease in BW and CFLW in the MUD treatment was further supported by the decrease in BCS, 12th rib BF, and RF throughout the treatment period. However, we did not observe differences in REA, plasma NEFA concentration, plasma cortisol concentration, or rectal temperature. Therefore, using the NASEM (2016) and the regression of CFLW for the heifers in the MUD treatment, we estimate that the muddy environmental conditions imposed on heifers increased net energy requirements by approximately 4.3 Mcal/day. This is greater than the estimated 3.9 Mcal/day for mature cows previously reported by Nickles et al. (2022). Although the heifers in both treatments were in a negative energy balance, it seems that they mobilized their own body tissues to provide nutrients and energy for sufficient fetal growth as there was no evidence of a treatment effect on calf birth weight. This indicates that during the last trimester, heifers will prioritize nutrients to fetal growth rather than maintenance and/or growth of their own tissues. As heifers in the CON treatment decreased their CFLW but increased their total maternal BW, it seems logical to conclude that those heifers had sufficient body stores at the start of the treatment period to provide for adequate fetal and placental growth to increase their total maternal BW. One would expect a gestating female that is growing a fetus to increase her total maternal BW as fetal weight is rapidly increasing as gestation progresses. However, heifers in the MUD treatment decreased both their CFLW and their total maternal BW while calf birth weight was not different from the CON treatment. It is possible that while heifers in the MUD treatment were also able to mobilize their body stores to provide for fetal growth, placental growth was negatively affected since heifers in the MUD treatment did not increase their total maternal BW during late gestation as one would expect of a gestating female. It is also important to remember that these heifers were not protein, vitamin, or mineral restricted, and were simply energy restricted. Furthermore, the efficiency of energy use seems to be similar between maintenance and fetal growth.

In addition, there was no evidence for an effect of treatment on calf plasma IgG concentration during the first 28 days of life, growth of calves up to weaning, or the percent of heifers cycling by the start of the breeding season. While it seems that the heifers used in the present study in both treatments were able to prioritize fetal growth and mobilize their own body stores, this may not be the case for heifers that do not start in as good of a body condition during late gestation. Therefore, the prioritization of nutrients may be dependent upon heifer BCS at the start of the last trimester when the majority of fetal growth is about to occur.

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## Conflict of interest statement

None declared.

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