

## **In utero heat stress effects on cows' cumulative milk yield**

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**The objective of this study was to determine whether in utero heat stress (IUHS) affects milk yield and lactation traits in Holstein cows in a hot environment. A total of 5278 cows with three consecutive lactations were used. Temperature-humidity index (THI) classes were defined as <76, 76-83 and >83 units. Average lactation length for first-lactation heifers was 415 days (SD=81) with no difference between IUHS heifers and non-IUHS heifers. First-calving heifers born to cows not exposed to heat stress (HS) during delivery produced 170 more kg of milk ( $P<0.05$ ) in 305-d lactations than heifers whose dams suffered HS (adjusted for the effects of THI one, two and three months previous to calving). Total milk yield for the first lactation was  $12477\pm 2828$  kg with no effect of THI at any stage of gestation. Heifers not exposed to IUHS at the day of delivery produced 0.7 more kg of milk daily compared to IUHS heifers at the day of delivery. Milk yield persistence was greater ( $P<0.05$ ) in first-calving heifers born to dams suffering heat stress at calving ( $73.5\pm 2.4\%$ ) compared to heifers born to HS cows at calving ( $72.9\pm 2.3\%$ ). IUHS at any stage of pregnancy did not influence subsequent cumulative total milk yield ( $37574\pm 5845$ ; across THI). It was concluded that there was evidence for altered milk yield and lactation persistence among IUHS first-calving heifers compared to non-IUHS heifers. However, these effects were less pronounced in subsequent lactations; thus, the IUHS effects on milk production did not go beyond the immediate impact in the first lactation.**

**KEY WORDS:** cumulative milk yield / extended lactations / in utero heat stress / lactation length / lactation persistency

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Maternal exposure to continuous high ambient temperatures during the last months of pregnancy has a direct impact on fetal development. During this period of rapid fetal growth, heat stress (HS) negatively affects both placental development and function [Limesand *et al.* 2018], which is reflected in lighter calves at calving [Tao and Dahl 2013, López *et al.* 2017]. HS leads to reduced placental mass and function [de Vrijer *et al.* 2004, Macko *et al.* 2013], which results in the development and progressive decline in fetal glucose and oxygen concentrations over the final third of gestation when fetal growth rate is at a maximum [Limesand *et al.* 2018, Rozance *et al.* 2018].

Mechanisms explaining placental and fetal growth constraints include the redistribution of blood to the body surface and reduced perfusion of the placental vascular bed [Dreiling *et al.* 1991, McCrabb *et al.* 1993]. Reduced placental function and mass increases vascular resistance in the placenta due to a reduction of angiogenesis by an abnormal expression of vascular endothelial growth factor and its receptors and placental growth factor [Regnault *et al.* 2002, Hagen *et al.* 2005]. This reduces glucose transport across the placenta [Thureen *et al.* 1992] by lower abundance of facilitated glucose transporters [Limesand *et al.* 2004, Wallace *et al.* 2005], slows growth of fetuses and alters their endocrine and metabolic profiles [Limesand *et al.* 2018].

Additionally, relative to calves born to cooled cows, calves from non-cooled dry cows present slower insulin clearance after insulin challenges [Monteiro *et al.* 2016a], while both passive and cell-mediated immune function of calves is also impaired by prenatal heat stress [Tao *et al.* 2019]. Thus, placental nutrient provision plays an important role in modulating maternal-fetal resource allocation, thereby affecting not only fetal growth, but altering the life-long health of the offspring [Fouden *et al.* 2006, Dimasuay *et al.* 2016].

Although evidence exists for epigenetic mediation of intergenerational effects in offspring from heat-stressed dams [Sinclair *et al.* 2016], it seems that prenatal heat stress undergoes adaptations in endocrinology, metabolism and organ functions, which alter the developmental programming and may have long-lasting effects throughout life. Pronounced interest is currently observed in the potential role of epigenetics in underlying the long-term effects of prenatal stress on the development of the fetus and these epigenetic changes in the fetus after prenatal stress have been documented in animal models [Jensen Peña *et al.* 2012]. This point is particularly important in dairy cattle, as in utero heat stress (IUHS) seems to alter the mammary gland microstructure and cellular processes during the cow's first lactation [Skibieli *et al.* 2018].

Although there is evidence that prenatal heat stress alters growth, the immune systems and metabolism of calves [Tao *et al.* 2012, Monteiro *et al.* 2016a], as well as milk production up to and through the first lactation of offspring [Monteiro *et al.* 2016b], and reproductive performance [Dahl *et al.* 2016], there is limited research, as yet, linking prenatal heat stress to milk yield in subsequent lactations of offspring. Additionally, studies that investigated the effects of IUHS on milk yield used only one lactation; therefore, it would be convenient to assess if hyperthermia during the fetal life affects subsequent milk yields during various lactations. Thus, this study aimed

to establish whether the temperature-humidity index (THI) experienced by dams at calving, one, two or three months previous to parturition could have carryover effects on the heifer's future milk production when considering multiple lactations.

## **Material and methods**

The research protocol was reviewed and approved by the Institutional Animal Care and Use Committee at the Autonomous Agrarian University Antonio Narro.

### **Farm and cows management**

Data were collected from one large commercial dairy farm (2800 cows) located in northeastern Mexico (25°N; 1120 m above sea level). Average annual temperature is 23.7°C and THI ranges from 70.1 to 89.8 units. Cows were housed in open dry-lot pens (161.5 m/cow) with shades (14.6 m/cow) in the center of each pen oriented north-south. Shade dimensions were 121.9 m long by 9.1 m wide by 4.0 m high. All animals had *ad libitum* access to feed and water and they were fed a total mixed ration balanced to meet or exceed nutrient requirements for prepartum Holstein cows weighing 650 kg (NRC 2001). Cows were fed two times daily a mixed ration with approximately 50% concentrate and approximately 5% leavings were removed immediately before each morning feeding.

Cows were inseminated after 50 days postpartum. Heifers and cows were observed for estrus three times a day, for approximately 30 min. Estrus detection was secured with the aid of pedometers and AI was conducted after visual observation of estrous behavior following the a.m. - p.m. guideline. Commercial sexed (for heifers) and non-sexed (pluriparous cows) frozen-thawed semen from multiple sires from the USA was used across all months of the year. Pregnancy was detected by palpation of the uterus per rectum about 45 days post-insemination. Services per pregnancy were 4.5, consequently lactation length was far beyond the traditional 10-11 months (>400 days).

### **Data collection**

A retrospective study was conducted using 5278 cows with three consecutive lactations (from 2015 to 2019). Information was obtained from the farm software and consisted of birth date, calving dates, parity, date of dry-off, 305-d milk yield, days in milk and total milk yield. Cows became eligible for the study when they completed three consecutive lactations, regardless of reproductive difficulties which lead to extended lactations in many of the cows. Additional criteria for inclusion of cows in the study were that they had not initiated their lactation with abortion or hormonal treatment. Cows were dried off 60 d before expected parturition or when milk yield reached 20 kg/day. Milk yield was measured electronically at each milking using the Dairy Comp 305 software (Valley Agricultural Software, Tulare, CA) for each cow.

For the duration of the study, meteorological data were obtained from a climatic station located 3 km away from the dairy operation. Maximum temperatures and

relative humidity were used to calculate the temperature-humidity index (THI; highest daily temperature in degrees Celsius; RH refers to maximum relative humidity) for each day using the following equation [Mader 2003]:

$$\text{THI} = [0.8 \times \text{temperature}] + [[\% \text{ RH}/100] \times [\text{temperature} - 14.4]] + 46.4$$

THI classes were defined as <76, 76-83 and >83 units. These cut-off values were based on THI values associated with changes in rectal temperature. For example, cows subjected to THI <76 show normothermia, whereas cows exposed to THI = 76 – 83 present rectal temperature  $\approx 40^{\circ}\text{C}$  and animals exposed to THI >83 present rectal temperature  $\geq 40.5^{\circ}\text{C}$  [Dikmen and Hansen 2009, Jeelani *et al.* 2019].

#### **Statistical analyses**

Milk production traits were analysed according to a completely randomized design with cow as the experimental unit. The statistical model included fixed effects of THI categories, with cow as a random effect. THI at calving, during the ninth, eighth and seventh month of pregnancy were included in the model, so that adjusted weather effects for particular months of gestation were calculated controlling for THI at different months of pregnancy. The computations were performed using the MIXED procedure of the SAS package programmes (SAS Institute Inc., Cary, N.C.)

Milk trait means were compared using the probability of a statistical difference (PDIF option of SAS). The occurrence of extended lactations was analysed using the PROC GENMOD of SAS. Statistical differences were considered significant at  $P < 0.05$ .

## **Results and discussion**

First-lactation milk yield data of Holstein heifers experiencing heat stress at delivery and previous to delivery (in utero) are summarized in Table 1. Average lactation length for first lactation heifers was 415 days (SD=81) with no difference between groups for THI groups. These lactation lengths are far above the traditional 305 days in milk for cows with a 12-13 month calving interval and were caused by the prolonged warm weather in the study site, which provokes a high number of services per pregnancy and consequently a prolonged days open period. Thus, these results suggested that maternal HS during pregnancy does not negatively affect offspring lactation length.

First-calving heifers born to cows not exposed to HS during delivery produced 170 kg more ( $P < 0.05$ ) milk in 305-d lactation than heifers, whose dams suffered HS at calving (adjusted for the effects of THI one, two and three months previous to calving). Likewise, compared with heifers born to cows exposed to HS during the eight-month of pregnancy, 305-d milk yield was higher ( $P < 0.05$ ) in heifers from dams not suffering HS or under HS two months before parturition (adjusted for THI at calving, one and three months before delivery). These results suggest a direct effect of IUHS on lactational physiology in first-lactation heifers and a carryover effect of

**Table 1.** The effect of temperature-humidity index (THI) in first-lactation Holstein heifers born to cows exposed to heat stress at calving or one to three months prior to parturition. Values are means (standard deviations in parenthesis)

| Variables                                    | THI<br>at calving        | THI<br>one month<br>before calving | THI<br>two months<br>before calving | THI<br>three months<br>before calving |
|--|--------------------------|------------------------------------|-------------------------------------|---------------------------------------|
| Lactation length 1 <sup>st</sup> lact (days) |                          |                                    |                                     |                                       |
| THI <76                                      | 412 (83)                 | 410 (77)                           | 413 (77)                            | 416 (84)                              |
| THI 76-83                                    | 416 (82)                 | 415 (85)                           | 414 (84)                            | 413 (80)                              |
| THI >83                                      | 418 (79)                 | 419 (79)                           | 417 (81)                            | 417 (82)                              |
| 305-d milk yield (kg)                        |                          |                                    |                                     |                                       |
| THI <76                                      | 9850 <sup>a</sup> (983)  | 9815 (1046)                        | 9803 <sup>a</sup> (1004)            | 9760 (1066)                           |
| THI 76-83                                    | 9680 <sup>b</sup> (1090) | 9768 (1031)                        | 9707 <sup>b</sup> (1102)            | 9790 (1028)                           |
| THI >83                                      | 9803 <sup>a</sup> (1191) | 9823 (1165)                        | 9850 <sup>a</sup> (1158)            | 9835 (1148)                           |
| Total milk yield 1 <sup>st</sup> lact (kg)   |                          |                                    |                                     |                                       |
| THI <76                                      | 12433 (2805)             | 12397 (2705)                       | 12469 (2873)                        | 12550 (2944)                          |
| THI 76-83                                    | 12437 (2983)             | 12495 (2959)                       | 12409 (2792)                        | 12429 (2824)                          |
| THI >83                                      | 12568 (2749)             | 12517 (2745)                       | 12533 (2788)                        | 12483 (2743)                          |
| Milk/day in 305-d lact (kg)                  |                          |                                    |                                     |                                       |
| THI <76                                      | 32.3 <sup>a</sup> (3.2)  | 32.1 (3.5)                         | 32.1 <sup>a</sup> (3.3)             | 31.9 (3.5)                            |
| THI 76-83                                    | 31.6 <sup>b</sup> (3.6)  | 31.9 (3.4)                         | 31.8 <sup>b</sup> (3.6)             | 32.0 (3.4)                            |
| THI >83                                      | 32.1 <sup>a</sup> (3.9)  | 32.2 (3.9)                         | 32.2 <sup>a</sup> (3.8)             | 32.2 (3.8)                            |
| Persistence 305-d milk yield (%)             |                          |                                    |                                     |                                       |
| THI <76                                      | 73.5 <sup>a</sup> (2.4)  | 73.4 <sup>a</sup> (2.7)            | 73.4 <sup>a</sup> (2.4)             | 73.1 (2.3)                            |
| THI 76-83                                    | 72.9 <sup>b</sup> (2.3)  | 73.3 <sup>a</sup> (2.6)            | 73.0 <sup>b</sup> (2.3)             | 73.3 (2.4)                            |
| THI >83                                      | 73.0 <sup>b</sup> (2.3)  | 73.1 <sup>b</sup> (2.3)            | 73.2 <sup>a</sup> (2.4)             | 73.3 (2.4)                            |

lact=lactation.

<sup>ab</sup>Within variables, means in the same column differ significantly at P<0.05.

IUHS on the calf's future milk performance. The present results are in line with results of Monteiro *et al.* [2016b] and Laporta *et al.* [2018], who observed that heifers born to late gestation HS cows had lower milk yields during their first lactations compared to heifers not suffering from IUHS.

This reduced milk yield of IUHS heifers during late pregnancy occurs because as cows undergo thermal stress in late gestation, so do the fetuses that must maintain core temperatures by adjusting blood flow and nutrient utilisation to limit heat production. This hyperthermia decreases the alveolar area of mammary glands of lactating heifers [Skibieli *et al.* 2018]. Given the positive association between the luminal area of mammary alveoli and the quantity of mammary secretory epithelial cells they possess, the lower the alveoli area, the lower the milk synthesis and storage, which appears to reduce milk production observed in IUHT cows. Another possible explanation for the reduced milk yield in IUHS heifers is related to epigenetic effects, because maternal HS during the dry period alters DNA methylation of the mammary gland during their first lactation [Skibieli *et al.* 2018, Reynolds *et al.* 2019]; therefore, these epigenetic marks due to IUHS may contribute to the reduced performance of the offspring in their adult life. Further, IUHS heifers have a lower percent of proliferating mammary cells [Skibieli *et al.* 2018] and present altered endocrine systems through early life that are consistent

with metabolic adaptations to accumulate energy in peripheral tissues and reduce lean growth. For instance, calves born to HS-dams present greater blood insulin in the first week of life relative to those born to cooled dams [Tao and Dahl 2013b]. Additionally, adiposity in puberty is related to subsequent milk yield [Silva *et al.* 2002]; therefore, the potentially greater accretion of fat in calves from HS dams could predispose those calves to alterations in mammary growth and reduced milk yield.

Due to the long lactations of first-calving heifers, total milk yield for the first lactation was 12477 kg (SD=2828) with no effect of IUHS at any stage of gestation. Heifers not exposed to IUHS at the day of delivery produced 0.7 more kg/day of milk compared to heifers exposed to IUHS at the day of calving. Surprisingly, daily milk yields of heifers suffering from severe IUHS at calving were not affected. These results were also found in animals exposed to IUHS two months previous to calving. Milk yield persistence was greater ( $P<0.05$ ) in first-calving heifers born to dams not suffering from heat stress at calving, and one and two months before calving compared to IUHS heifers at calving and one or two months before calving.

For greater milk production and efficiency it is desirable to have cows with high lactation persistency. In the present study, IUHS heifers presented a marginal, but significant reduction in lactation persistence. These results further suggest that IUHS alters mammary growth throughout postnatal life and possibly restrict the maintenance of the number and activity of milk-secreting cells and increased cell death via apoptosis with advancing lactation, which ultimately reduced lactation persistence in IUHS cows.

None of the milk variables were affected by IUHS during the seventh month of pregnancy. In developmental programming, the timing and duration of stressors will influence particular responses [Reynolds *et al.* 2019]. Hence, the windows to IUHS reflected in subsequent milk yield seem to be active during the eighth and ninth month of pregnancy. Growth of bovine fetuses follows an exponential trend with the maximal rate of body weight gain by 7.6 months [Prior and Laster 1979]. Thus, biological changes in the mother during the most active growth of the fetus were involved in fetal programming induced by prenatal stress, which altered subsequent milk yield of offspring.

Maternal HS exposure during calving as well as at one, two or three months before calving did not influence subsequent cumulative days in milk, total milk yield (sum of three lactations), and percentage of extended lactations (>500 days) during three consecutive lactations (Tab. 2). This is contrary to observations of Laporta *et al.* [2018], who reported that granddaughters born to late gestation heat-stressed dams produced less energy-corrected milk in their second lactation compared with granddaughters born to cows without heat stress during the dry period. This discrepancy appears to be due to the extended lactations practiced in the present study, prolonging the cumulative days in milk and milk yield, which completely compensated for the milk reduction by IUHS, by the additional milk yield per lactation. The reduction in milk yield in IUHS cows likely resulted from irregular mammary morphology as a result of alterations

**Table 2.** The effect of temperature-humidity index (THI) experienced by first-calf Holstein heifers at delivery and previous to delivery (in utero) on the subsequent cumulative total milk yield (kg) during three consecutive lactations. Values are means (standard deviations in parenthesis)

| Variables                          | THI<br>at calving | THI<br>one month<br>before calving | THI<br>two months<br>before calving | THI<br>three months<br>before calving |
|------------------------------------|-------------------|------------------------------------|-------------------------------------|---------------------------------------|
| Days in milk, sum 3 lact (days)    |                   |                                    |                                     |                                       |
| THI <76                            | 1249 (191)        | 1243 (1870)                        | 1250 (193)                          | 1254 (197)                            |
| THI 76-83                          | 1248 (199)        | 1254 (201)                         | 1250 (197)                          | 1249 (194)                            |
| THI >83                            | 1259 (196)        | 1257 (189)                         | 1257 (192)                          | 1255 (192)                            |
| Total milk yield, sum 3 lact (kg)  |                   |                                    |                                     |                                       |
| THI <76                            | 37497 (5751)      | 37357 (5681)                       | 37524 (5804)                        | 37632 (5937)                          |
| THI 76-83                          | 37449 (5987)      | 37655 (6022)                       | 37512 (5919)                        | 37497 (5844)                          |
| THI >83                            | 37772 (5879)      | 37639 (5734)                       | 37712 (5760)                        | 37626 (5781)                          |
| Lactations >500 d (%) <sup>1</sup> |                   |                                    |                                     |                                       |
| THI <76                            | 12 (272/2337)     | 11 (134/1248)                      | 12 (137/1196)                       | 13 (167/1305)                         |
| THI 76-83                          | 13 (204/1557)     | 13 (326/2544)                      | 12 (309/2523)                       | 11 (256/2248)                         |
| THI >83                            | 11 (157/1384)     | 12 (173/1486)                      | 12 (187/1559)                       | 12 (210/1725)                         |

lact = lactation.

<sup>1</sup>Values in parenthesis are number of cows with extended lactations/total.

in the development of the fetal mammary gland [Skibieli *et al.* 2018]. However, the extended lactations of cows in the present study possibly stimulated more renewal of mammary epithelial cells throughout lactation, which would result in higher secretory activity. This is so because the absence of pregnancy during lactation suppresses secretion of estrogens, thus increasing milk yield and preventing the increased mammary epithelial cells loss [Herve *et al.* 2016].

## Conclusion

These data provide compelling support for the view that prenatal heat stress, particularly during the eighth and ninth months of pregnancy, can have long-lasting effects on milk yield at maturity of in utero heat-stressed heifers. However, the extent of this milk loss due to late gestation heat stress is less pronounced and disappears over three consecutive lactations. Thus, under the present conditions of exposure to heat stress for most of the year, the reduced milk yield caused by in utero heat stress may be completely compensated by improved health and the adoption of extended lactations, which would increase cow longevity and cumulative milk yield.

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