Spanish Journal of Agricultural Research
21 (2), e0501, 13 pages (2023)
eISSN: 2171-9292
https://doi.org/10.5424/sjar/2023212-19600
INIA-CSIC

RESEARCH ARTICLE

OPEN ACCESS

Effect of heat stress and body condition score on the occurrence of puerperal disorders in Holstein cows

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Abstract

Aim of study: To evaluate the association between temperature-humidity index (THI) and body condition score (BCS) at calving and retained placenta (RP), puerperal metritis, clinical ketosis, and mastitis in Holstein cows in a hot environment.

Area of study: Northeastern Mexico.

Material and methods: This is a retrospective cohort study (n= 12,102 lactations from January 2017 to December 2021) using univariate logistic regressions. The outcome variables were periparturient diseases, and the predictor variables were BCS and thermal stress at calving.

Main results: Cows calving with a THI > 82 were 30% more likely (prevalence 16.8% vs 13.7%; p < 0.01) to have RP than cows whose parturition occurred with moderate or low thermal stress (THI < 82 units). Cows calving with THI > 82 had significantly increased chances of having metritis than cows calving with THI < 82 (prevalence 15.6 vs 13.4; p < 0.01). Cows calving with a THI > 82 were 1.8 times more likely to have clinical ketosis (7.6% vs 4.4%; p < 0.01) than cows calving with THI < 82 units. Cows with BCS at calving ≥ 3.5 had half the risk of having RP (prevalence 10.4 vs 19.1%, p < 0.01) than cows with BCS < 3.5. Likewise, the risk of metritis decreased (p < 0.01) with BCS ≥3.5 at calving (prevalence 10.9 vs 17.4%).

Research highlights: Heat stress at calving was associated with an increased risk for RP, puerperal metritis, and clinical ketosis compared to cows undergoing mild or no heat stress at parturition. Also, cows with BCS ≥ 3.5 were less likely to present RP and metritis, but high body fatness was associated with an increased risk for clinical ketosis.

Additional key words: temperature-humidity index; body energy reserves; retained placenta; metritis; ketosis; mastitis.

Abbreviation used: BCS (body condition score); BHBA (β-hydroxybutyric acid); NEFA (non-esterified fatty acid); OR (odds ratio); RP (retained placenta); THI (temperature-humidity index); TMR (total mixed ration).

Citation: Mellado, M; Herrera, CD; De Santiago, A; Veliz, FG; Mellado, J; García, JE (2023). Effect of heat stress and body condition score on the occurrence of puerperal disorders in Holstein cows. Spanish Journal of Agricultural Research, Volume 21, Issue 2, e0501. https://doi.org/10.5424/sjar/2023212-19600

Received: 25 May 2022. **Accepted:** 25 Apr 2023.

Funding agencies/institutions	Project / Grant
Autonomous Agrarian University Antonio Narro, Saltillo, Mexico	3001-2419

Competing interests: All authors declare no actual or potential conflicts of interest between the authors and other people or organizations that could inappropriately bias their work.

Availability of data: The data supporting this study's findings are available from the corresponding author, J.E.G, upon reasonable request.

Introduction

In addition to the metabolic heat production (i.e., body heat) by dairy cows which increases in parallel with increased milk yield (Yan et al., 2021), heat is also gained from the environment. In zones with hot weather, constant high ambient temperatures cause an increase in body temperature and induce a physiological response (Hao et al., 2016). In subtropical and tropical regions, an almost constant high ambient temperature, far above the thermoneutral environment of dairy cattle, predominate throughout the year (Li et al., 2020), which reduces milk production of dairy cattle by 40% to 60% respect to temperate conditions (Usman et al., 2013). Furthermore, dairy cows without supplemental cooling activate various adaptive mechanisms to increase the external net energy flow, such as elevated rectal temperatures, respiration rates, skin temperatures, heart rates, and reduced rumination time (Garner et al., 2017; Yan et al., 2021). Thus, ambient temperature is strongly and negatively correlated with production responses (e.g., milk yield, milk temperature, and feed intake) in dairy cows (Amamou et al., 2019; Ji et al., 2020). Air temperature also severely depresses the reproductive performance of cows (Mellado et al., 2013) and weakens the immune system (Bagath et al., 2019), resulting in increasing puerperal diseases with increasing the temperature-humidity index (THI) (Gernand et al., 2019).

An adequate body condition score (BCS) is important for maintaining the health status of dairy cows (Dale et al., 2017). Therefore, optimizing BCS at calving is an essential aspect of the dry period management for improving the subsequent lactation (Zhao et al., 2019), reproductive performance (Bedere et al., 2018), and reducing periparturient diseases (Dubuc et al., 2010; Torres et al., 2020). The recommended acceptable BCS of dairy cows ranges from 2.5 to 3.5 on a 5-point scale to ensure that periparturient health is not compromised (Giuliodori et al., 2013). Unfortunately, despite the existence of various reports on the effect of heat stress and BCS in the transition period on the occurrence of periparturient diseases for several European cattle breeds in temperate zones, not enough efforts have been made so far (at least to our knowledge) to assess these associations in regions with high ambient temperature for most of the year.

Therefore, given that several studies dealing with the effect of BCS at calving for cows reared under different cooling systems on dairy production and metabolic disease incidence are few and sometimes conflicting, the motivation for this study was to quantify the effect of BCS and THI on the occurrence of some calving-derive diseases in high-yielding Holstein cows in a hot environment. Hence, the current study hypothesized that low BCS and high ambient temperature at calving are associated with a higher incidence of retained fetal membranes and metritis in high-yielding Holstein cows. Furthermore, it was hypothesized that increased BCS at calving results in a higher prevalence of clinical ketosis. Therefore, one objective of the current study was to evaluate the associations between BCS at parturition and the occurrence of various calving-derived diseases in multiparous Holstein cows in a hot environment. Additionally, the current study aimed to determine the effect of thermal stress at calving on the occurrence of periparturient illness.

Material and methods

Animals, feeding, and facilities

Cows used in this study were handled following the guidelines defined by the 'Guide for Care and Use of Agricultural Animals in Research and Teaching' (https://www.fass.org/images/science-policy/Ag_Guide_3rd_ed.pdf). Likewise, this study was authorized by the Autonomous Agrarian University Antonio Narro Animal Care Advisory Committee (approval number 5-5-30-38111-4250-3001-2419).

Holstein cows from a single commercial dairy farm (≈4500 milking animals) in northern Mexico (25° N, elevation 1145 m, mean annual rainfall 234 mm, mean annual temperature 23.7 ºC) were included in this study. Cows were housed in open dirt pens with adequate shade.

Cows were fed a total mixed ration (TMR) based on alfalfa hay, corn silage, and grain concentrate (cottonseed meal, soybean meal, corn grain, and a mineral premix) twice daily in approximately equal quantities at two meals, at ≈08:00 and 16:00 hours. The forage-to-concentrate ratio was 50:50, and this ration met the requirements of fresh Holstein cows weighing 650 kg and producing 38 kg of 3.5% fat-corrected milk (NRC, 2001). TMR was subsequently adjusted according to milk production (0–30, 30–150, 150–210, and 210 to ≥305 days in milk). Cows were given ad libitum access to water and were fed ad libitum with about 5% feed refusal; the residual feed was removed at 07:00 h every day.

On the day of calving, cows received a calcium bolus containing calcium formate, calcium propionate, calcium chloride, magnesium chloride, calcium gluconate, potassium chloride, magnesium sulfate, copper sulfate, potassium iodide, 1-2 propanediol, nicotinamide (Calcibol®, Nutrilag, Gomez Palacio, Durango, Mexico). Also, on the day of calving, cows received 300 mL of propylene glycol. Cows were milked three times daily at 01:00, 09:00, and 17:00 h. BCS (1 to 5 scale, 0.25 points increment; Ferguson et al., 1994) was evaluated at calving by the same trained observer.

Study design and disease recording

This retrospective observational cohort study included 12,102 lactations from January 2017 to December 2021.

Table 1. Effect of heat stress and body condition score at calving on the occurrence of retained placenta, metritis, and clinical ketosis in high-yielding Holstein cows from a single herd in a hot environment.

THI at calving	Incidence (%)	Odds ratio (OR)	95% CI OR	p
	Retained placenta			<0.0001
≥82	631/3762 (16.8)	1.3	1.1–1.4	
<82	1146/8340 (13.7)	Reference		
	Metritis			0.0012
≥82	588/ 3762 (15.6)	1.2	1.1–1.3	
<82	1118/ 8340 (13.4)	Reference		
	Ketosis			0.0001
≥82	286/3762 (7.6)	1.8	1.5–2.1	
<82	363/8340 (4.4)	Reference		
BCS at calving				
	Retained placenta			<0.0001
≥3.5	638/6143 (10.4)	0.49	0.4–0.5	
<3.5	1139/5959 (19.1)	Reference		
	Metritis			<0.0001
≥3.5	667/ 6143 (10.9)	0.58	0.5–0.6	
<3.5	1039/ 5959 (17.4)	Reference		
	Ketosis			0.0222
≥3.5	358/ 6143 (5.8)	1.2	1.0–1.4	
<3.5	291/ 5959 (4.9)	Reference		

THI= temperature-humidity index; CI= confidence interval. BCS= Body condition score (1 to 5 scale scoring system, 0.25 points increment). The reference category is one, and there is no confidence interval for that.

The dairy farm veterinarian diagnosed puerperal metritis starting at day 4 until 20 after calving; cows were monitored for puerperal metritis twice weekly by recording the thickness of the uterine wall by rectal palpation and the observation of abnormal vaginal secretion associated with watery purulent reddish-brownish fetid vaginal discharge containing flecks of pus within the first 15 d postpartum (Sheldon et al., 2006). Retained placenta (RP) was diagnosed based on the presence of fetal membranes protruding from the vulva for over 24 h after calving. Clinical ketosis was diagnosed by the herd veterinarian based on reduced milk yield, decreased feed intake, and loss of body condition associated with a positive urine strip test for ketone bodies (Ketostix®, Bayer Mexico, CDMX, Mexico). Based on these signs, ketosis was deemed clinical. Using the first streams of milk from all quarters, the California mastitis test was used regularly to detect clinical mastitis. Results were recorded as 0 (negative), trace (slight reaction), 1 (mild reaction), 2 (moderate reaction), or 3 (strong reaction).

Climatic data were obtained from a meteorological station located 2.5 km away from the dairy operation for the duration of the study. Information registered was daily maximum temperatures and relative humidity. The air temperature was recorded with a mercury thermometer under full shade and 1.5 m above the ground. This information was used to calculate the daily temperature-humidity index (THI), using the following equation (highest daily temperature in Celsius degrees; RH refers to maximum relative humidity):

$$THI = (0.8 \times \text{temperature}) + ((\% \, RH/100) \times (\text{temperature} - 14.4)) + 46.4.$$

Following the THI categories proposed by Collier et al. (2012), THIs are classified as thermoneutrality (THI < 72), mild-moderate heat stress (72 < THI < 79), moderate-severe heat stress (82 = THI < 89), and severe heat stress (THI > 90).

Statistical analysis

Logistic regressions were performed using the LOGISTIC procedure in SAS (SAS Inst. Inc., Cary, NC, USA) to assess the effects of thermal condition at calving THI at calving < 82 vs > 82 units) and body condition score at parturition (< 3.5 vs ≥ 3.5 units) on the presence or absence of RP, puerperal metritis, clinical ketosis, and mastitis. Initially, a multivariable logistic regression was performed to model the effects of thermal stress, BCS at calving, and the thermal stress × BCS interaction, with the individual animal

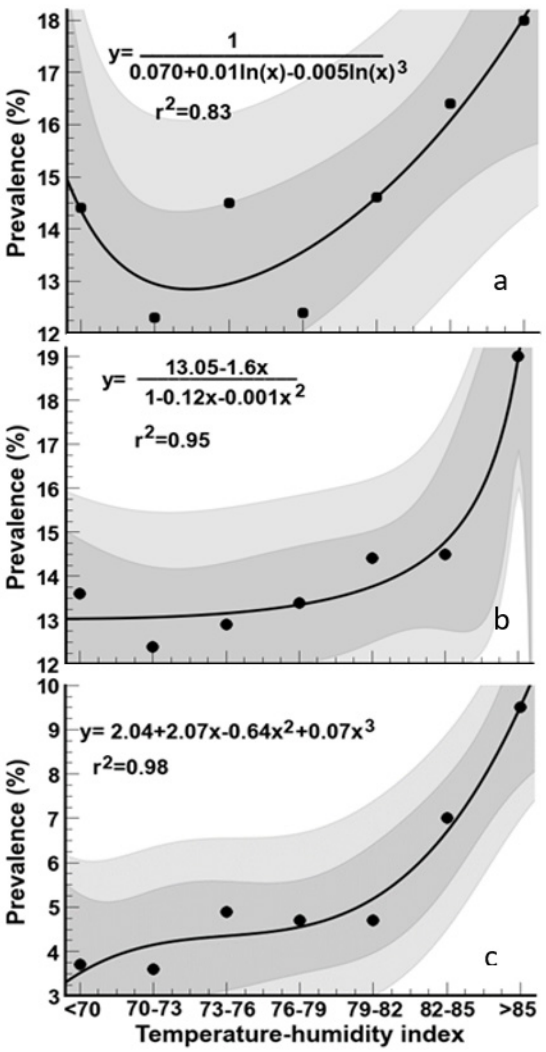

Figure 1. Distribution of the lactation prevalence of retained placenta (a), puerperal metritis (b), and clinical ketosis (c) in multiparous Holstein cows from a single herd, undergoing various temperature-humidity indexes at calving. The association was based on 12,102 lactations from January 2017 to December 2021 in a hot environment. Darker bands are 95% confidence intervals for predicted values. Lighter bands are 95% confidence intervals for real values.

as the observational unit. Given that thermal stress × BCS interaction was non-significant, this term was deleted, and univariate logistic regressions were performed to assess the effect of single independent variables to estimate odds ratios and 95% confidence intervals, incorporating lactation number as covariate. The association between various THI at calving and the prevalence of periparturient diseases was assessed using the CurveExpert Professional 2.5.6 software (Hyams Development, Madison, AL, USA). The same procedure was used to evaluate the association between different BCS scores at calving and the prevalence of the studied diseases. Values with $p < 0.05$ were regarded as statistically significant for all statistical analyses.

Results

Of the 12,102 calvings evaluated, 1,777 (14.8%) ended with a RP. The overall lactational prevalence of puerperal metritis and clinical ketosis was 14.1 and 5.4%, respectively. Incidence rate of clinical mastitis was 0.28 cases per 365 cow-days at risk, and both heat stress and BCS were not significantly related to this disease. The logistic regression model results for the 12,102 lactations that had complete data (all categorical variables) are presented in Table 1. Compared to cows calving with thermoneutrality or mild-moderate heat stress (THI < 82), cows calving with moderate to severe heat stress (THI > 82), were 1.3 more likely to have RP. The relationships between THI at calving and the prevalence of RP were curvilinear. The occurrence of this reproductive disorder varied little with THI < 82, after which RP augmented at an increasing rate (Fig. 1a). Cows undergoing thermoneutrality or mild-moderate heat stress at calving had lower odds ($p < 0.01$) of suffering puerperal metritis than did cows suffering heat stress at calving. According to Fig. 1b, the regression models highlighted that the breakpoint for the distinctive increase in the prevalence of puerperal metritis was THI > 85 (moderate to severe heat stress).

Moderate to severe heat stress at parturition was associated with an increased risk of clinical ketosis than cows calving with low or mild to moderate heat stress. The cubic relationships between THI at calving and the prevalence of clinical ketosis showed that the occurrence of this metabolic illness varied slightly with THI < 82, after which clinical ketosis augmented at an increasing rate (Fig. 1c). There was no THI × BCS interaction in the initial multivariate model for all diseases.

Estimated odds ratios (OR) and their 95% confidence intervals for BCS associated with puerperal diseases are given in Table 1. Cows with BCS at calving greater than 3.5 had half the risk of presenting RP than thinner cows at calving (Table 1). Fig. 2a shows that RP was progressively lower for cows with greater BCS at calving. Likewise, the risk of puerperal metritis was greater in cows with BCS < 3.5 at calving (Fig. 2b). Conversely, BCS greater than 3.5 at calving increased the risk of clinical ketosis compared to cows with BCS < 3.5 at calving. The trends for the prevalence of puerperal metritis and clinical ketosis by BCS at calving are given in Fig. 2b and 2c. The prevalence of puerperal metritis remained somewhat similar with BCS < 3.25, but after this value, the prevalence of puerperal metritis plummeted. Conversely, the association between BCS at calving and prevalence of clinical ketosis followed a curvilinear trend with the highest prevalence in cows with the highest body energy reserves at calving.

Discussion

The prevalence of RP observed in this study was well within the normal range (9.0-15.4%) reported across dif-

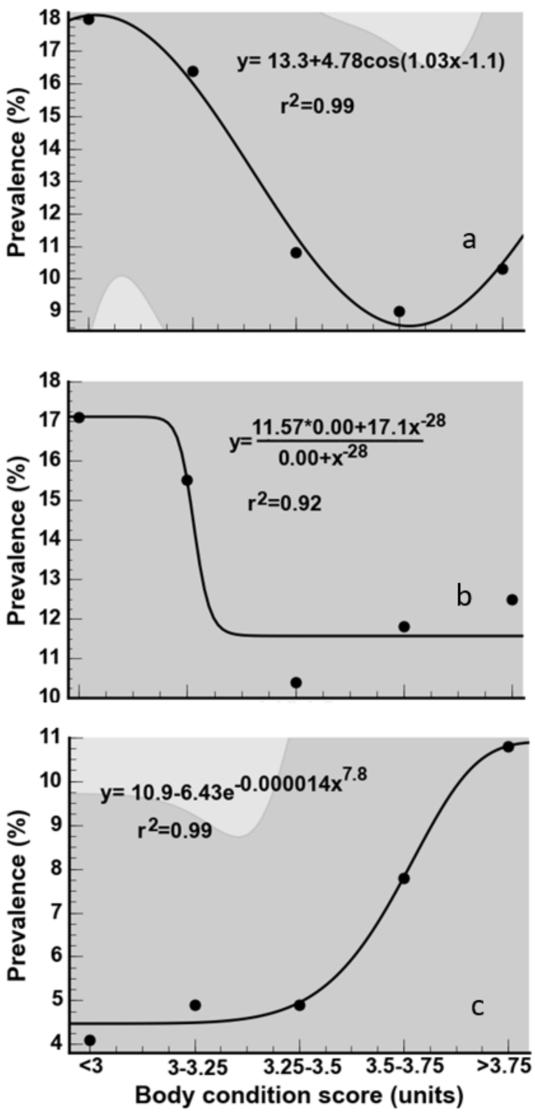

Figure 2. Distribution of the lactation prevalence of retained placenta (a), metritis (b), and clinical ketosis (c) by body condition score (BCS) at calving in pluriparous Holstein cows from a single herd in a hot environment (n=12,102). Darker bands are 95% confidence intervals for predicted values. Lighter bands are 95% confidence intervals for real values.

ferent countries, breeds, and management systems (Buso et al., 2018; Mahnani et al., 2021). In the current study, the lactation prevalence of metritis is consistent with various studies that have reported 18 to 40% (Giuliodori et al., 2013; Genís et al., 2018). This range suggests that the incidence of puerperal metritis is not a biologically fixed rate and that differences in herd management may allow some control of the incidence of this infectious disease. The observed prevalence of clinical ketosis in this herd was below the range of reported data (11.6-23%; Berge & Vertenten, 2014; Vanholder et al., 2015). This marked difference seems to arise from using different cow-side tests to detect ketone bodies.

Many risk factors have been identified for RP (Gilbert, 2016), including heat stress (Ahmadi & Mirzaei, 2006; Gernand et al., 2019). In agreement with the findings of the present study, Bahri Binabaj et al. (2014) observed higher incidences of RP in warm seasons. However, these results may be confounded with the effect of photoperiod because more extended photoperiod in summer months is associated with decreased immune function than that of shorter day length (Thompson & Dahl, 2011). In addition, Gernand et al. (2019) and Menta et al. (2022) documented an increase in RP with increasing THI in the periparturient period in commercial farms. In contrast, other studies have reported more cases of RP in the cold season (Hossein-Zadeh & Ardalan (2011), or calving season or heat stress in the periparturient period has not been associated with the incidence of RP (Yeon-Kyung & Ill-Hwa, 2005). Different ambient temperature and humidity ranges or management systems among studies may account for these contrasting results. In the current study, the maximum THI was 97.4, which illustrates the severity of the cows' heat load. This great discomfort presumably triggered physiological changes in cows undergoing heat stress which impacts oxidative stress, hormonal imbalance, and immune responses (Hashem & Amer, 2008; Chauhan et al., 2021) and may hinder the ability of cows to detach the placenta adequately.

Data from the current study demonstrated that severe heat stress was an important predisposing factor for having puerperal metritis, which agrees with Gernand et al. (2019), who observed a positive relationship between metritis and an increase in THI in the five days after calving in dairy cows. Also, puerperal metritis or purulent vaginal discharge incidences have been higher in spring and summer-calving cows than in autumn and winter-calving cows (Giuliodori et al., 2017; Jeong et al., 2018). The evidence indicates that as temperature and humidity rise, there are effects on host response to the pathogen microbiome, causing metritis. High ambient temperature causes immune suppression, resulting in increased risk of disease incidence (Lacetera, 2019) due to increased multiplication and distribution of microbes (Quintana et al., 2020). Additionally, higher metritis incidences under hot-humid conditions could be due to the multiplication of pathogen carriers, such as flies.

On the contrary, Benzaquen et al. (2007) reported that cows that calved during the warm season had lower puerperal metritis than those calving during the cool season. These contrasting results could be explained by different management strategies to alleviate heat stress, proper hygienic practices, heat stress intensity, and uterine microbiota dynamics in postpartum dairy cows. Of interest, there has been no association of the THI in the previous week to calving on metritis, suggesting that the immune response in the early postpartum period is highly sensitive to heat stress.

The current study revealed a cause-effect connection between high THI at calving and a markedly increase in the prevalence of clinical ketosis, which is consistent with Jeong et al. (2017), who observed that cows that calved during the summer had a higher risk of ketosis than cows that calved during other seasons. However, contrary to these findings, other studies have found a greater incidence of ketosis in cows calving in winter or cold weather (Mellado et al., 2018). Differences in climate, the intensity of heat stress, geographical location, number of observations, methods used to determine ketosis, cooling systems in dairy barns, and diet characteristics would explain the considerable inconsistencies in the effect of weather conditions at calving on the occurrence of clinical ketosis. In the current study heat-stressed cows at calving, ketosis could be due to the confounding effects of decreased feed intake (Pérez-Báez et al., 2019).

Thin cows at calving were more prone than cows with BCS ≥ 3.5 to have RP, which is in accordance with Ghaffari et al. (2019) and Qu et al. (2014). The current study suggests that lower BCS may precede RP. One potential reason for the higher prevalence of RP in cows with BCS < 3.5 is lower feed intake at calving, as has been documented by Dervishi et al. (2016). This is reflected in higher fatty acids, non-esterified fatty acids (NEFA), and β-hydroxybutyric acid (BHBA) concentrations than in healthy cows (Cellini et al., 2019; Yazlık et al., 2019). High blood NEFA concentration during the last seven days before calving has been identified as a risk factor associated with greater chances of developing RP (LeBlanc, 2008). Moreover, these high serum NEFA levels, which determine the occurrence of negative energy balance and metabolic stress, cause depression in the immune system, inhibiting rejection and expulsion of fetal membranes and causing their retention (Mordak & Stewart, 2015).

Cows with a BCS < 3.5 at calving were more likely to have metritis. Findings of this study support results from previous studies (Dubuc et al., 2010; Kadivar et al., 2014) where thin cows (BCS ≤ 2.75) at parturition increased the odds of developing cytological endometritis or metritis. There is a positive association between prepartum blood NEFA concentration or BCS loss and the incidence of postpartum metabolic and infectious diseases, and elevated prepartum blood NEFA is associated with an increased risk of metritis (Dubuc et al., 2010). The relationship between BCS, feed intake, and the occurrence of metritis has been described in the literature and can be explained by the detrimental effects of a negative energy balance on the immune system (Huzzey et al., 2007). High serum BHBA concentration in the first-week post-calving has been associated with a 2.2-fold increase in metritis (Duffield et al., 2009; Torres et al., 2020). The mechanism explaining why cows diagnosed with metritis had elevated serum BHBA concentrations could be that they were suffering a poor adaptive response to the onset of lactation and the resulting negative energy balance. The effect of elevated serum BHBA levels on metritis risk seems to be mediated by the impacts of hyperketonemia on immune function (Zarrin et al., 2014).

In the present study, high BCS at calving was associated with an increased risk of clinical ketosis, which agrees with findings in high-yielding dairy cows (Shin et al., 2015; Vanholder et al., 2015). Over-conditioned high-yielding cows at calving have more severely depressed feed intake postpartum, leading to a more pronounced negative energy balance. Subsequently, they are more susceptible to mobilized greater body energy reserves shortly before calving, which results in higher blood NEFA and BHBA levels (Nogalski et al., 2012), leading to hyperketonemia (Guliński, 2021). In addition, due to many cows in the dairy operation studied, failures in the formation of production groups may have caused some cows to receive inadequate rations for their lactation stages. Thus, cows may have been overfed at the end of their lactation and enter the dry period over-conditioned, which in turn would increase the risk for clinical ketosis.

This study confirms the detrimental effect of heat stress at calving on health of high-yielding Holstein cows. Cows subjected to heat stress at parturition were more likely to present RP, puerperal metritis, and ketosis. Also, this study revealed that an adequate body condition score at calving was associated with lower odds for RP and puerperal metritis, but cows with high body energy reserves at calving (BCS ≥ 3.5) are particularly linked to an increased risk of clinical ketosis. These findings highlight the importance of heat stress abatement mechanisms for cows approaching calving in hot climates to reduce periparturient diseases.

Authors' contributions

Conceptualization: M. Mellado
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Formal analysis: M. Mellado
Funding acquisition: M. Mellado, J.E. García
Investigation: C.D. Herrera, A. de Santiago, F.G. Véliz
Methodology: J. Mellado
Project administration: M. Mellado
Resources: Not applicable
Software: J. Mellado
Supervision: M. Mellado, J.E. García
Validation: J. Mellado, A. de Santiago
Visualization: J. Mellado, A. de Santiago, F.G. Véliz
Writing – original draft: M. Mellado
Writing – review & editing: M. Mellado, C.D. Herrera, F.G. Véliz, J.E. García

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