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ANIMAL REPRODUCTION

Environmental and physiological measures in the neonatal period as indicators of growth and puberty of Holstein heifers

João Vitor Nogueira de Almeida[1] **Letícia Ribeiro Marques**[1] **Angélica Cabral Oliveira**[1] **Tiago do Prado Paim**[1] **Thaisa Campos Marques**[1] **Karen Martins Leão**[1*]

[1]Programa de Pós-graduação em Zootecnia, Instituto Federal de Educação, Ciência e Tecnologia Goiano, 75901-970, Rio Verde, GO, Brasil. E-mail: karen.leao@ifgoiano.edu.br. *Corresponding author.

ABSTRACT: The present study assessed the influence of environmental variables at birth (birth season, temperature, relative humidity, THI - temperature and humidity index) and newborn serum protein level of 450 Holstein heifers on growth (body weight, and mortality rate) and reproductive performance (weight and age at puberty, age at first conception, and conception rate at first artificial insemination). The immune passive transfer was positively related to the weights at 30 and 60, with a trend at 90 days, which consequently affected weight at puberty, age at puberty, and age at conception. Therefore, it reinforces the importance of protocols with newborns that allow the assessment of the passive transfer of immunity. Calves born during the warmer months exhibited 4.2 and 12.28 kg less weight at weaning and puberty, respectively, compared to calves born during winter. Thus, calves born in an environment with higher THI showed changes in development, health, and reproductive efficiency in the short, medium, and long term. These findings reinforce the importance of adopting better colostrum management and welfare conditions, especially in warm season, to allow successful immune passive transfer and increase system efficiency.
Key words: immune passive transfer, serum protein, birth season, temperature and humidity index, body weight.

Medidas ambientais e fisiológicas no período neonatal como indicadores de crescimento e puberdade de novilhas Holstein

RESUMO: O presente estudo teve como objetivo avaliar a influência das variáveis ambientais ao nascimento (estação de nascimento, temperatura, umidade relativa, ITU - índice de temperatura e umidade) e nível de proteína sérica no recém-nascido de 450 novilhas Holandesas sobre o crescimento (peso corporal e taxa de mortalidade) e o desempenho reprodutivo (peso e idade na puberdade, idade na primeira concepção e taxa de concepção na primeira inseminação artificial). A transferência de imunidade passiva foi positivamente relacionada ao peso corporal aos 30, 60 e 90 dias, o que consequentemente afetou o peso na puberdade, idade na puberdade e idade na concepção. Isto reforça a importância de protocolos com recém-nascidos que permitem a avaliação da transferência passiva de imunidade. Os bezerros nascidos durante os meses mais quentes apresentaram 4,2 e 12,28 kg de peso a menos no desmame e na puberdade, respectivamente, em comparação aos bezerros nascidos durante o inverno. Assim, os bezerros nascidos em um ambiente com maior THI apresentaram alterações no desenvolvimento, saúde e eficiência reprodutiva a curto, médio e longo prazo. Esses achados reforçam a importância de adotar melhores condições de manejo e bem-estar do colostro, especialmente nas estações mais quentes, para permitir a transferência de imunidade passiva bem sucedida e aumentar a eficiência do sistema.
Palavras-chave: transferência de imunidade passive, proteína sárica, estação de nascimento, índice de temperatura e umidade, peso corporal.

INTRODUCTION

Animal welfare assessment must be based on three different aspects: the environment, the animal's physiological response, and signs indicating failure of homeothermic condition (HERBUT et al., 2018). Environmental conditions adversely affect animal production systems by hindering their productive and reproductive potential (SOUZA et al., 2010).

The heat stress (HS) damage in lactating cows is already well known but other stages of their development need more research. Moreover, the birth period is essential for immune system development and is dependent on the early consumption of high-quality colostrum produced during the last 2 to 4 weeks of pregnancy (MCGRATH et al., 2016, BAUMRUCKER et al., 2022).

Challenges on the dam during the dry period compromise fetal growth, immune function performance, and calf development (TAO et al., 2012; OUELLET et al., 2020). For example, prenatal heat stress impairs the passive transfer of colostral IgG (TAO et al., 2012), accelerates the jejunal enterocyte apoptosis at birth, compromises immune organ growth, and reduces growth relative to calves born to cooled dams (AHMED et al., 2021).

We hypothesized that the environmental conditions at birth and neonatal care could

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determine the heifer's development up to the first conception. The present study verified the influence and associations of environmental conditions and physiological measures at birth on heifer development and reproductive efficiency.

MATERIALS AND METHODS

Housing and management

We performed an observational study using data from January 1 of 2016 to December 31 of 2018 of a commercial dairy farm in Santa Helena de Goias, Goias, Brazil. Holstein cows and heifers (n = 450) with 245 ± 7 days of gestation were housed in a compost barn production system. Fans positioned in the bed and feeding area were activated at temperatures above 20 °C and turned off at temperatures below 17 °C and the sprinklers were turned on twice a day (10 am and 3 pm) for 40 min (at 1-min on and 7-min off cycle) to provide thermal comfort. Total mixed ration (TMR), comprising corn silage, soybean meal, ground corn, cottonseed, and a commercial ration, was offered ad libitum twice a day, following the recommendations of the National Research Council (NRC, 2001).

Soon after birth, the calves received 3.80 ± 2.27 L of high-quality colostrum (>21.0% Brix corresponding to least 50.0 g/L of Ig/mL according to QUIGLEY et al. (2013), corresponding to approximately 10% of the born body weight (38.96 ± 4.81 kg). Twenty-four hours after birth, blood samples were collected from the jugular vein in an untreated 4 mL vacuum tube and attached to a 21 G needle (25 × 8 mm). The tube was kept at room temperature until blood serum and clot separated. After, serum was evaluated in a Brix refractometer (Instrutemp®, Belenzinho, Brazil), at a Brix scale from 0° to 32° Brix, to determine the classification for immune passive transfer according to the methodology used by LOMBARD et al. (2020): excellent (≥9.4%, corresponding to ≥25.0 g of IgG/L), good (8.9 – 9.3%, corresponding to 18.0 – 24.9 g of IgG/L), fair (8.1 – 8.8%, corresponding to 10.0 – 17.9 g of IgG /L), and poor (<8.1%, corresponding to < 10.0 g of IgG /L).

The calves were fed according to age: 0–30 days (4 L of milk, water, and concentrate with 18% protein ad libitum), 31–70 days (6 L of milk, water, and concentrate ad libitum), 71–80 days (4 L of milk, 3 kg of concentrate, 3 kg of corn silage, and water ad libitum), 81–90 days (2 L of milk, 3 kg of concentrate, and silage and water ad libitum). After weaning, the total mix ration (TMR) comprising corn silage, soybean meal, ground corn, and commercial base mix (mineral and vitamin) was provided by the recommendations of the National Research Council (NRC, 2001). The diet was offered ad libitum twice a day. The females were weighed at birth, 30, 60, and 90 days using a digital scale (Coimma®, Dracena, Brazil).

Calves were kept in individual pens in a shed until 30-days old, and then, they were sent to collective paddocks with 15 animals each until weaning, at 90 days of age. All animals were dewormed monthly. Diseases data (diarrhea, pneumonia, babesiosis, and anaplasmosis) that occurred from birth until weaning were collected from Ideagri® (Rehagro, Belo Horizonte, Brazil) program. Thereafter, the females remained confined and housed in contemporary groups, until they reached puberty and body weight higher than 330 kg.

Puberty was determined by the presence of corpus luteum in the ovary by weekly ultrasound scanning, when they received 25 mg (i.m.) of dinoprost tromethamine (5.0 mL of Lutalyse, Zoetis, São Paulo, Brazil). Artificial insemination was performed 12 hours after estrus detection. Pregnancy was diagnosed using transrectal ultrasonography (DP-2200, Mindray®, São Paulo, Brazil) 30 days after insemination.

Environmental variables

Environmental variables (temperature and relative humidity) were collected from the weather station located on the farm (ADAMA Clima®, Adama Brasil, Londrina, Brazil). Temperature and humidity index (THI) was calculated for the calving dates of each cow using the maximum, minimum, and average temperatures, according to the model defined by MADER et al. (2006): $ITU = 0.8 \times T + [(RH (\%) \div 100) \times (T-14.4)] + 46.4$, where T is the temperature in degrees Celsius and RH is the relative humidity. The birth seasons were defined by the dates corresponding to the seasons of the year in the southern hemisphere: spring (21 September to 20 December), summer (21 December to 20 March), autumn (21 March to 20 June), and winter (21 June to 20 September).

Statistical methods

First, outliers, normality of residuals, and homogeneity of variance were checked. Data were transformed to a logarithmic scale when necessary. Data analysis was divided into two datasets: before and after weaning. Data were analyzed using the R software v.4.1.0 (R CORE TEAM, 2021).

Mixed model analysis was used to assess the influence of the season of birth on the continuous traits evaluated as summarized in table 1. The "lme4",

Table 1 - Final mixed models evaluating each response variable.

Response variable	Fixed effects	Random effects	P-value
Gest Length	Birth Season + Nlact	Cow; Bull	< 0.05
Wbirth	Birth Season + NLact + Gest Length	Cow; Bull	< 0.05
W30 W60	Birth Season + NLact + Wbirth + SerumPtn	Cow; Bull	< 0.05
W90	Birth Season + NLact + Wbirth + SerumPtn + Ndiseases	Cow; Bull	< 0.05
SerumPtn	Birth Season + NLact + Vcolostrum + ColostrumQ	Cow; Bull	< 0.05
Puberty Age Puberty Weight PregAge NAI	Wbirth + SerumPtn + Wweaning	Bull	< 0.05

Gest Length = gestation length, Wbirth = weight at birth, W30 = weight at 30 days, W60 = weight at 60 days, W90 = weight at 90 days, SerumPtn = serum protein, Puberty Age = age at puberty, Puberty Weight = weight at puberty, PregAge = age at pregnancy, NAI = number of artificial insemination, Birth Season = season of birth, Nlact = lactation number, Ndiseases = number of diseases, Vcolostrum = volume of colostrum, ColostrumQ = quality of colostrum, Wweaning = weight at weaning.

“car”, and “emmeans” packages were used for analyzing the mixed models and for comparing the least-squares means using Tukey test. No significant variables were removed from the models, choosing the best model for each response variable was based on the lowest Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC).

Binary traits were analyzed through logistic regression analyses using “car”, “lme4”, and “broom.mixed” packages. Moreover, the influence of birth season, lactation order, number of diseases, and serum protein levels on mortality up to weaning were evaluated. Birth season’s impact on mortality from weaning to the first parturition was also evaluated. The influence of birth season, weight at 90 days, semen type (conventional or sexed), and type of AI synchronization (“estrus” or “estrus and ovulation” protocols) on conception rate at first AI were analyzed.

The Wald test was used to assess the significance of the effects in the logistic regression model. The appropriate models to explain the behavior of each response variable were selected based on AIC and BIC.

“FactoMineR” and “Factoextra” packages were used for principal component analysis. A graphical approach based on the explained proportion of variance for each eigenvector was applied using Cattell´s rule, which determines that the components corresponding to the eigenvalues to the left of the horizontal line must be retained (the eigenvalues corresponding to random variables are on a line parallel to the x-axis). The graphs were plotted using the “ggplot2” package.

Discriminant analysis was performed in SAS® University Edition using the PROC DISCRIM and PROC STEPDISC. The discriminant analysis aimed to understand if the animal data correctly classify the observation into the birth season and which variables were important for this classification. Statistical significance was considered when $P < 0.05$ and to suggest a trend toward significance when $0.05 < P < 0.1$.

RESULTS

During the experimental period, the highest mean temperature (24.99 ± 0.25 ºC) and THI (74.53 ± 0.39) were presented in spring. RH record was maximum in summer (79.70 ± 1.10%) and minimum in winter (47.05 ± 1.21%). These data are typical in tropical regions with rainy summer and dry winter.

Suckling phase

In this study, the length of gestation revealed an interaction between the season of the year in which parturition occurred and the lactation number of the cow ($P = 0.006$). The primiparous cows that calved in spring revealed shorter gestation length (269 ± 1.98 days) compared to those that calved in winter (278 ± 1.73 days) and second lactation (278 ± 1.92 days) and third lactation (283 ± 2.51 days) cows that calved in the spring ($P < 0.05$).

Calf birth season did not have a significant effect on birth weight ($P = 0.71$), while the number of lactation had a significant effect ($P = 0.03$). Calves from third lactation cows revealed higher birth weight (39.0 ± 0.78 kg) than the first lactation calves (37.1 ± 0.64 kg; P = 0.003), but no differences were reported for calves from second and more than three lactation (P > 0.05).

Calves born in the summer exhibited lower weight at 30 days (2.3 kg) compared to those born in winter (P = 0.01; Table 2). Those born in spring weighed 3.4 kg more at 60 days than those born in winter (P = 0.009; Table 2). The weight at 90 days of calves born in summer was 4.2 kg lower than those born in winter (P = 0.02; Table 2). Moreover, the weight at 30 days was positively influenced by serum protein levels estimated by the Brix refractometer (P < 0.0001).

The heaviest calves at 60 days were more commonly daughters of third-lactation cows than of first-lactation cows (P = 0.006). However, there were no significant differences in weight at 60 days from the second lactation or cows in their fourth or later lactation (P > 0.05). Furthermore, birth weight (P < 0.0001) and serum protein levels (P = 0.019) influenced the weight at 60 days. Serum protein levels tended to influence the body weight at 90 days (P = 0.10), similar to that observed at 30 and 60 days. Additionally, higher body weight at 90 days was related to fewer diseases since birth (P = 0.05). The lactation number of the cow and calf birth season did not affect the mortality rate from birth to weaning (P > 0.05).

Principal component analysis and discriminant analysis of the suckling phase

The first two dimensions explained 32.3% of the variance observed in data from calves until weaning. The environment variables (RH, THImin, and THImax) contributed significantly to the first dimension. THImin, THImean, SerumProt, Vcolostrum, Birth Weight, and W90 contributed to the second dimension.

Birth weight was positively correlated with the lactation number and gestation length (Figure 1). THI data revealed opposite results to weight data, with lower THI being associated with higher weights (Figure 1). Furthermore, relative humidity was negatively correlated with serum protein levels.

Data from calves until weaning was able to classify the observations into birth season correctly only in 57.9% of the cases in winter, 53.1% in summer, 39.3% in spring, and 33.3% in autumn. Therefore, the better discriminatory power of the data was seen for observations from winter and summer. Spring and autumn data had higher misclassification into summer and winter, respectively. The variables that contributed significantly to this classification were serum protein levels; calf weight at 30, 60, and 90 days; and the number of diseases from birth to weaning.

Rearing phase

Calves born in the summer weighed 12.28 kg less when they reached puberty than those born in the winter (P = 0.013; Table 3). Weight at puberty was influenced by weight at weaning (P = 0.02) with a positive correlation (r = 0.06), it was not influenced by serum protein levels (P = 0.13). The conception rate at the first AI was not influenced by calf birth season (P = 0.41; Table 3). Although, the age at puberty was influenced by weaning weight, it was not directly influenced by birth season and serum protein levels (P > 0.05). Age at 1st conception decreased with higher weaning weight (P = 0.0593). On the other side, age at 1st conception was not associated with the birth season and serum protein levels (P > 0.05).

Heavier calves at 90 days tended to have a higher conception rate at the first AI (P = 0.08). Calf birth season did not influence the mortality rates from weaning to puberty (P > 0.05).

Principal components and discriminant analysis of the rearing phase

The first four dimensions of PCA explained together 54.0% of the variance. The first dimension was represented mainly by THI data. The second dimension was related to age at 1st conception, number of artificial inseminations to conception, birth weight, and weight at 90 days. The third dimension was related to RH, THImin, serum protein, birth weight, and the number of artificial inseminations to conception.

Table 2 - Mean and standard error of body weight of heifers at 30, 60, and 90 days according to the season.

Heifer weight	Spring	Summer	Autumn	Winter
30 days	55.6 ± 0.8ab	53.4 ± 0.8b	54.6 ± 0.8ab	55.7 ± 0.9 a
60 days	73.8 ± 1.3a	71.8 ± 1.3 ab	70.7 ± 1.3ab	70.4 ± 1.4b
90 days	101.0 ± 1.9ab	97.6 ± 1.9b	101.0 ± 1.9ab	101.8 ± 2.0a

a,b Different letters on the same row are statistically different (P < 0.05).

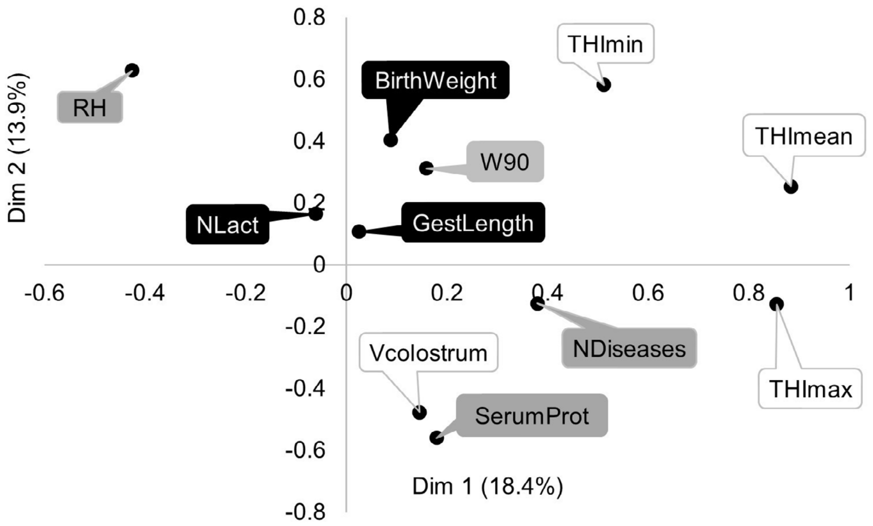

Figure 1 - Coordinates of variables in first and second dimensions of principal components analysis using data of calves from birth to weaning. Birth weight was positively correlated with the lactation number and gestation length, lower THI were associated with higher weights, and relative humidity was negatively correlated with serum protein levels.

*RH = relative humidity, THImin = temperature index and minimum humidity at parturition, THImean = temperature and humidity index mean at parturition, THImax = maximum temperature and humidity index at parturition, Nlact = lactation number, GestLength = pregnancy length, Wbirth = calf weight at birth, W30 = calf weight at 30 days of life, W60 = calf weight at 60 days of life, W90 = calf weight at 90 days of life, SerumPtn = serum protein, Ndiseases = number of diseases until weaning.

Therefore, we decided to look at variable coordinates in the second and third dimensions (Figure 2).

High birth weight and weight at 90 days were associated with decrease in age at puberty (Figure 2). An inverse association was observed between serum protein levels and reproductive variables (age at pregnancy and number of AIs), with higher levels of serum protein being associated with a lower age at pregnancy and the number of AIs.

The discriminant analysis was able to appropriately classify observations into winter and summer, in 45.3% and 46.9% of cases, respectively. Only weight at puberty and age at pregnancy were entered in the final discriminant model. Accuracy of classification in the fall and spring was remarkably lower, 11% and 25.8%, respectively.

A second discriminant analysis was performed to determine whether the entire data set could discriminate between heifers that became pregnant in the first AI and those who did not. The final discriminant model classified "yes" correctly in 56.8% of cases and "no" in 59.04% of cases.

Table 3 - Mean and standard error of weight at puberty (kg), age at puberty (days), age at conception (days), and conception rate at first service artificial insemination (Conception Rate-%) according to calf birth season.

Variable	Spring	Summer	Autumn	Winter
Weight at puberty	357±3.3ab	350 ± 3.1b	353 ± 3.2ab	362 ± 3.5a
Age at puberty	414±6.7a	400 ± 6.4a	403 ± 6.4a	408 ± 7.0a
Age at conception	492±11.9a	496 ± 10.3a	473 ± 9.7a	459 ± 10.7a
Conception rate	33.3%a	35.4%a	45.7%a	44.0%a

a,b Different letters on the same row are statistically different ($P < 0.05$).

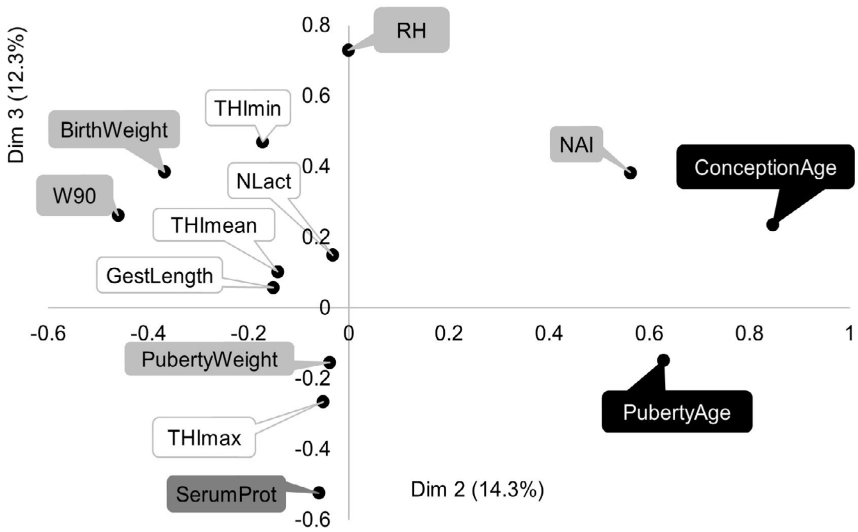

Figure 2 - Coordinates of variables in second and third dimensions of principal components analysis using data of calves from weaning to the first conception. High birth weight and weight at 90 days were associated with decrease in age at puberty, while higher levels of serum protein were associated with a lower age at pregnancy and the number of AIs.

*RH = relative humidity, THImin = minimum temperature and humidity index at parturition, THImean = mean temperature and humidity index at parturition, THImax = maximum temperature and humidity index at parturition, NLact = lactation number, Birth Weight = calf weight at birth, W90 = calf weight at 90 days of life, NAI = number of artificial inseminations until conception, SerumPtn = serum protein after birth, Puberty Age = age at puberty, Puberty Weight = weight at puberty, Conception Age = age at conception.

This model was composed only by serum protein level. Therefore, serum protein level was associated with the probability of the heifer becoming pregnant in the first AI.

DISCUSSION

This study presents the influence of environmental and physiological variables at birth on the development and reproduction of heifers. Then, we evaluated the relationship between variables with principal component analyses of data from each heifer's growth and puberty phase and the discriminatory power of these variables with discriminant analyses. Environmental conditions affect the development and reproductive efficiency of dairy cattle, thereby triggering significant losses to the system. Such losses were identified as reduced development, health, and reproductive efficiency, as the calves underwent small changes in the THI on the day of their birth.

The birth weight did not differ during various seasons of the year. This may have occurred because of the comfort environment promoted to cows during the prepartum period (compost barn production system with fans and sprinklers). According to OUELLET et al. (2020), calves born to late-gestation heat-stressed dams are on average 4.6 kg lighter than calves born relative to calves that were born to dams in a comfort zone thermal. However, the birth weight increased when the gestation length of the cow was longer. This may be due to the interaction between the season of the year in which the birth occurred and the mother's lactation number (P = 0.006).

The results obtained for weight at 30, 60, and 90 days and serum protein levels support our hypothesis that the development of calves born in warmer seasons such as spring and summer, with higher temperatures and average THI, is reduced and leads to a series of losses until weaning. Heat stress is more intense in summer because of high temperatures with high humidity. In winter, the low humidity of the air ends up reflecting in better thermal comfort.

This study revealed significant differences in the body weight of calves born in summer compared to those in winter in the first 30 days of life (53.4 ± 0.79 kg vs. 55.7 ± 0.85 kg). The weaning weight of calves born in the summer was lower than those born in the winter (97.6 ± 1.9 kg vs. 101.8 ± 2.03 kg). These data can be associated with the differences in THImean (73.14 ± 0.36 vs. 71.45 ± 0.39) and RH (RH: 79.70% ± 1.10% vs. 47.05% ± 1.21%) observed between summer and winter, respectively.

Serum protein levels positively influenced the weight at 30 and 60 days and tended to influence the weight at 90 days. Passive transfer of IgG, serum antioxidant concentrations, growth index, and intestinal development are markedly dependent on colostrum quality ingested on the day of birth (YANG et al., 2015). However, the ability of calves to absorb IgG from the ingested colostrum is adversely affected by HS (TAO et al., 2012). Thus, calves born in winter reveal better immune system compared to those born in summer and, consequently, greater weight at 30 days.

According to previous studies (TAO et al., 2012; MONTEIRO et al., 2016), calves with reduced weight have less efficiency in converting feed to output, reduced immunity; and consequently, higher mortality rates. Our findings showed that weight at puberty was influenced by weight at weaning, albeit demonstrated a weak positive correlation. However, it is important to note that feeding and management during the transition from milk to solid feed influence the development of a healthy gut and rumen (KHAN et al., 2016) and this can affect different aspects of development in calves. However, dairy heifers should be fed and managed to achieve 55% of their mature body weight (BW) at first breeding. Thus, efficient colostrum ensures adequate serum protein levels and thus leads to better immunity, health, and development of the calf. Notably, HS causes short-term changes in the immunological and physiological function of calves, leading to reduced weight and health, which corroborates the results found by TAO et al. (2012) and MONTEIRO et al. (2016).

The reduced performance caused by HS at the beginning of the calf life extends until the rearing stage, as the weight at weaning, at 90 days of life, was lower in summer than that in winter ($P < 0.01$). Notably, calves born in the summer exhibited lower weight at puberty (350 kg). This difference reveals the long-term damage that HS can cause since animals in the summer were in a medium-moderate HS zone (mean THI = 73.14 ± 0.36). Calves born in seasons with milder environmental variables tend to be heavier, have a more robust immune system, and better reproductive performance compared to those born in an HS environment, as in the summer period, confirming our hypothesis that the season of the year of calf birth interferes in your development and reproductive performance as heifer.

CONCLUSION

The birth season of calves can affect their development, health, and reproductive efficiency. In tropical regions with rainy summer and dry winter, animals born in the summer were affected by high average temperature and humidity, with lesser development of the calves and lower weight at heifer puberty. It is important to consider that associations are findings of an observational study where passive transfer of immunity was estimated and not measured by serum total protein, which can lead different responses in other systems and environment. Although, these limitations, adopting better colostrum and welfare management is important independently of birth seasons to allow the successful immune passive transfer. Moreover, a deeper understanding of the factors that interact with nutrition and immunity at each developmental stage creates opportunities for developing strategies to improve immune responses and future cattle performance.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors critically reviewed the manuscript and approved the final version.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

This is an observational study. The Goiano Federal Institute Ethics Committee has confirmed that no ethical approval is required.

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