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Comparison of milk production and calving intervals between Slick and Wild-Type Holsteins in a tropical grazing production system

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Abstract. Heat stress is a significant challenge to dairy production in most parts of the world. Puerto Rican Criollo cattle carry the Slick (SL) gene that results in short lustrous hair, conferring them with superior heat tolerance that makes them a valuable genetic resource in dairy breeding programs. The SL gene has been part of the Puerto Rican dairy cattle genetic pool long enough that we have registered SL Holsteins with ancestry percentages greater than 93 %. We documented milk production throughout the lactation cycle and calving intervals (CI) of SL registered Holstein cows in El Remanso Dairy Farm, in Camuy, Puerto Rico (18.4839° N, 66.8450° W). Official production and reproductive data from the Dairy Records Management System (www.drms.org) was obtained and used to compare the production of 17 SL hair registered Holstein cows with 68 wild-type (WT) Holsteins. Milk production was analyzed using Proc GLIMMIX of SAS (SAS University Edition, 2018) and a Tukey test was conducted to analyze milk production during the hot period using a model that included genotype (SL and WT), stage of lactation, and lactation number (1, 2 or ≥ 3) as fixed effects. Tukey test analysis were also performed comparing the CI between 4-12 SL and 4-12 WT Holsteins with data from 2013-2016 (number of cows varied by year). Average milk production for SL and WT Holsteins was 16.59 ± 0.94 and 14.83 ± 0.41 kg/day ($p = 0.746$). SL Holsteins showed a shorter CI than their WT contemporaries with an average of 14.42 ± 0.13 versus 16.06 ± 0.08 ($p = 0.001$). Under the hot and humid conditions of Puerto Rico, SL Holstein dairy cows perform reproductively better than WT Holsteins. Therefore, the SL gene may be an appropriate adaptive strategy to support an efficient dairy industry within a warmer global climate.

Key Words: Dairy Cattle, Milk production, Calving interval, Slick Gene, Holstein

Comparación de la producción de leche y los intervalos entre partos entre vacas Holstein Pelonas y No-Pelonas en un sistema de producción a pastoreo en un clima tropical

Resumen. El estrés por calor es un reto para la producción de leche en la mayor parte del mundo. El ganado Criollo puertorriqueño es portador del gen Slick (SL) que resulta en un pelo corto lustroso que le otorga mejor tolerancia al calor, lo que los hace un recurso genético valioso en programas de mejoramiento genético de ganado lechero. El gen SL ha sido parte del acervo génico de ganado lechero puertorriqueño tiempo suficiente y tiene hatos con ganado Holstein SL registrados con porcentajes de ascendencia > 93 %. Se evaluó la producción de leche a través del ciclo de lactancia y los intervalos entre partos de vacas Holstein registradas en la vaquería El Remanso en Puerto Rico. Los datos productivos y reproductivos se obtuvieron del Dairy Records Management System (www.drms.org), para comparar la producción de 17 vacas Holstein SL registradas con 68 Holstein fenotipo silvestre (WT). Se utilizó Proc GLIMMIX de SAS (SAS University Edition, 2018) y una prueba de Tukey para analizar la producción de leche durante el período caluroso utilizando un modelo que incluía genotipo (SL y WT), etapa de lactancia y número de lactancia como efectos fijos. La prueba de Tukey también se realizó comparando el intervalo de parto (CI) entre 4-12 SL y 4-12 WT con datos de 2013-2016 (n varía según el año). La producción promedio de las SL fue 16.59 ± 0.94 kg/día, mientras que las WT produjeron un promedio de 14.83 ± 0.41 kg/día($p=0.746$). Las SL mostraron un CI más corto que las WT con un promedio de 14.42 ± 0.13 vs 16.06 ± 0.08 ($p = 0.001$). Bajo condiciones climáticas de Puerto Rico las vacas lecheras SL tienen un mejor rendimiento reproductivo que las WT. El gen SL puede ser una estrategia adaptativa para apoyar una industria lechera eficiente en un clima global más cálido.

Palabras claves: ganado lechero, producción de leche, intervalo entre partos, gen “Slick”, Holstein

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Introduction

Heat stress causes economic losses of around $897 million dollars in the dairy industry across the United States (St-Pierre *et al.*, 2003) due to a productive and reproductive decline. The Slick (SL) Holstein cattle have been documented to have lower rectal temperatures under heat stress conditions, therefore is regarded as more tolerant to high temperatures than Wild-type Holsteins (Curbelo *et al.*, 2016; Ríos-Solís *et al.*, 2019a). Slick Holsteins have been documented to produce higher milk yields and present shorter calving interval (CI) than their wild-type (WT) contemporaries (Pantoja *et al.*, 2005).

Elevated environmental temperature, coupled with increased humidity, and the metabolic heat from milk production results in higher body temperatures (West, 2003). When the body heat surpasses 25 ºC, the capacity of dairy cattle to dissipate heat diminishes (Roenfeldt, 1998. Kadzere *et al.*, 2002, Bernabucci *et al.*, 2010. Belhadj *et al.*, 2016), which results in less dry matter intake and milk production (West, 2003). Additionally, heat stress negatively affects animal's reproductive ability by hindering gamete development, pre-implantation embryonic development and conceptus growth in early lactation (Hansen, 2009).

Selecting for thermotolerant traits in cattle can be an important tool to counterpart the negative effect of increasing temperatures. Belhadj *et al.*, (2016) defined heat tolerance as "ability of the animals to maintain the expression of their inherited functional potential when raised under hot conditions". For over 500 years, farmers in Puerto Rico have been selecting the more adaptable animals to the tropical environment (Molina-Fernández, 2001). Since mid-twentieth century, farmers in Puerto Rico have been crossing their criollo cattle with Holstein cattle, generating SL-hair Holstein cattle with registered ancestry percentages greater than 93 %; resulting in highly productive cattle adapted to a hot environment.

Observational studies using Dairy Records Management Systems data have suggested that SL Holstein have higher milk productions than WT Holsteins in tropical conditions (Pantoja *et al.*, 2005; Ortiz-Colón *et al.*, 2018). However, when data from SL and WT dairy cows were used to compare the milk production of both genotypes throughout the years, factors such as stage of lactation and lactation number of the cows have not been considered. We hypothesized that under heat stress conditions SL Holstein cows would present higher milk yield than WT cows, independent of lactation number, at all stages of lactation. To test this hypothesis, milk production data taken during the hot period (March-August) of 2015 in a farm located in Puerto Rico was analyzed. The temperature-humidity index (THI) was used to measure cow heat stress level during the trial; CI data was also analyzed to assess whether the observed differences between SL and WT Holsteins were significant.

Material and Methods

Animals, feeding and handling:

Production data of 43-68 WT Holstein and 5-17 SL Holstein cattle (animal numbers varied depending on test date) was obtained to compare the performance of both studied phenotypes (Figure 1). El Remanso Dairy Farm has the largest number of registered SL Holsteins in Puerto Rico and the data collected for the study corresponds to all available animals at the farm. All dairy cows were on a semi-intensive rotational grazing system based on *Cynodon nlemfuensis* and milked twice a day (10:00 and 22:00). Animals were supplemented with 4.54 kg of a commercial dairy concentrate at each milking. According to chemical analysis, this feed contained 19 % crude protein, 13.3 % ADF, 28.5 % NDF, NFC 44.9 %, crude fat 4.6 %, 5.83 % ash and a NEL of 1.78 Mcal/kg MS. Moreover, microscopic analysis of the feed revealed that it was composed of 39 % wheat middling's, 25 % ground yellow corn, 14 % soybean meal, 11 % corn distillers dried grains, 9 % oat hulls, 1.4 % limestone, and 0.6 % salt. In addition, the feed contained 50 grams of monensin per 909 kg of dry matter.

Location and climate conditions:

The study was conducted using official records from a commercial dairy farm located in Camuy, Puerto Rico (18.4839° N, 66.8450° W; Figure 2). Temperature and relative humidity corresponding to the period of interest was obtained from a meteorological station (Isabela Agricultural Experimental Station 18°46′30″N 67°05′13″W) located 19 km west of El Remanso Dairy Farm (Figure 2) to determine the level of heat stress experienced during the trial, using the THI. THI was calculated using the formula:

$$THI = (1.8*AT+32)-[(0.55-0.0055*RH)\times(1.8*AT-26)]$$

where AT =air temperature (ºC), and RH = relative humidity (%).

Maximum and minimum temperature (°C), relative humidity (%) and THI (average included) can be observed in Table 1. Following Zimbelman *et al.*, (2009) thresholds for heat stress, cows in this study, on average, experienced mild to moderate heat stress during the experimental timeline (March to August). The minimum THI shows that the cows are under the threshold of heat stress, corresponding to the coolest hours of the day such as dawn and mornings. In contrast, the maximum THI shows that lactating cows are under moderate (78-89) heat stress, reaching severe (≥ 89) heat stress in August. The mean THI ranged from 72.29 to 78.49, putting cows under mild (68-78) heat stress all year long. After analyzing the climatological data from the study period, we observed other months with higher THI, but the studied months were selected based on average climatological data of two additional HOBOlink meteorological stations located on the northern and southwest zones of Puerto Rico (Gurabo Agricultural Experimental Station – 18.2518°N, 65.9904°W and Lajas Agricultural Experimental Station – 18.0321°N, 67.0736°W).

Figure 1. Images depicting the phenotype of the Wild-type Holstein (A) and the Slick Holstein (B). Absence of long hairs on the poll can be observed in the Slick Holstein.

Figure 2. Location of el Remanso Dairy Farm in Camuy, Puerto Rico (marked in red), and location of the meteorological station in the Isabela Agricultural Experimental Station (yellow star).

Table 1. Monthly minimum and maximum temperatures and relative humidity from 2015 in Isabela Puerto Rico and monthly maximum, minimum and average THI calculated.

	Temperature (°C)		% Relative humidity		Temperature humidity index		
Month	Min	Max	Min	Max	Min	Max	Mean*
JAN	17.8	28.7	40.5	97.2	62.0	83.3	72.3 ± 3.1[a]
FEB	17.8	28.7	40.5	96.6	62.0	83.2	72.3 ± 3.1[a]
MAR	18.4	29.3	48.5	98.4	63.1	84.4	72.8 ± 3.3[b]
APR	19.3	29.2	42.9	96.9	63.8	84.2	74.2 ± 2.9[c]
MAY	21.0	30.9	59.9	99.6	67.1	87.6	75.7 ± 2.9[e]
JUN	20.5	30.5	62.1	97.3	66.6	86.4	77.4 ± 2.9[f]
JUL	21.3	31.3	50.2	97.2	66.8	87.8	77.9 ± 2.7[g]
AUG	22.1	33.1	59.9	96.9	68.7	91.0	78.3 ± 2.9[gh]
SEP	21.5	32.9	54.7	98.6	67.5	90.9	78.5 ± 3.1[h]
OCT	21.8	31.3	59.4	97.4	68.3	88.0	77.3 ± 2.9[f]
NOV	19.5	29.7	60.2	99.0	65.0	85.3	75.6 ± 3.1[de]
DEC	20.1	31.8	60.0	97.8	65.8	88.9	75.2 ± 3.03[d]

*THI means with different small letter superscripts differ significantly ($P < 0.05$)

Data collection and statistical analysis:

Official production data from March to August of 2015 was obtained from the Dairy Records Management System (DRMS; www.drms.org) to compare milk yields among the genotypes. During the study period, the average THI levels ranged from 72.3 to 78.3, indicating mild to moderate heat stress, respectively. The data collected included daily (test day) milk production, lactation number and days in milk (DIM). Cows reproductive efficiency was evaluated using CI as used by previous researchers (Grosshans *et al*., 1997; Sattar *et al*., 2005). Calving interval data from 2013 (n=4 WT and n=4 SL), 2014 (n= 11 WT and n=11 SL), 2015 (n= 12 WT and n=12 SL) and 2016 (n= 12 WT and n=12 SL) was also collected from DRMS.

Milk production data (dependent variable) was analyzed using the Proc GLIMMIX procedure in SAS (SAS University Edition, 2018). The fixed effects used in the statistical model included: genotype (SL and WT), stage of lactation (fresh, peak, mid, late and too-late) and lactation number (1, 2, or ≥ 3); whereas cow ID was used as a random variable. Multiple comparison procedures were made applying an adjusted Tukey test. Stage of lactation was categorized according to DIM as fresh (1-39 DIM; n=13 WT and n=4 SL), peak (40-100 DIM; n=13 WT and n=9 SL), mid (101-199 DIM; n=32 WT and n=10 SL), late (200-305 DIM; n=39 WT and n=8 SL) and too-late (>305 DIM; n=41 WT and n=11 SL). The CI data was analyzed using a Proc GLIMMIX in which all pairwise differences were evaluated with an adjusted Tukey test. The statistical model used was the following:

$$Y_{ij}=\mu+\alpha_i+\beta_j+\varepsilon_{ij}$$

where μ=mean effect, α=effect of genotype, β= year and ε= residual.

Results and Discussion

Milk production

The milk yield data between SL and WT was compared after considering the stage of lactation and lactation number, and no differences were found in milk yield during the hot period (p = 0.766). Through all periods of lactation, only numerical differences (P < 0.05) among WT and SL cows were found; early lactation (WT=23.45 ± 3.37 vs SL=18.45 ± 3.68 kg/d), peak lactation (WT=25.95 ± 2.04 vs SL=23.64 ± 3.93kg/d), mid-lactation, (SL= 21.97 ± 2.12 vs WT=18.73 ± 0.79 kg/d), late-lactation (SL=16.46 ± 1.19 vs WT=14.18 ± 0.44 kg/d) and the end-lactation (SL=11.49 ± 1.00 kg/d vs WT=10.59 ± 0.36 kg/d). Conversely, stage of lactation had a significant (P < 0.0001) effect on milk production during the hot period (Figure 3). As expected, cows had a higher milk yield during the peak and fresh stages of lactation, following by mid, late, and too-late consecutively. Meanwhile, no significant effect (p= 0.063) was observed, yet a tendency of number of lactations was found. It is plausible that this is due to the low animal number analyzed and thus it is hypothesized that with a larger sample a statistical difference may be found.

Dikmen *et al*., (2014) reported that cattle calving during the summer season had a lower milk yield during the first 90 days than cattle calving during the winter; these differences were lessened in SL cattle; However there was no significant difference in milk production based on genotype (SL or WT) alone, which concur with the findings in this study. Also, another study performed by our research team showed no differences in milk production nor feed efficiency between SL and WT dairy cows (Ríos-Solís *et al*., 2019b). However, in another study, milk production data of Carora × Holstein cattle (75 % Holstein, 25 % Carora) of different hair length was analyzed by Olson *et al* (2003) and showed that SL Carora × Holstein cattle had higher milk yield. Because cows in Puerto Rico are, on average, under heat stress throughout the year, this could explain why no differences in milk yields were found between genotypes during the studied period because both genotypes are acclimatized to heat stress. Therefore, we proposed that the putative milk production advantage of SL cows previously documented in dairy farms in Puerto Rico is the result of shorter CI of SL cows which results in SL herds having less DIM at any given point.

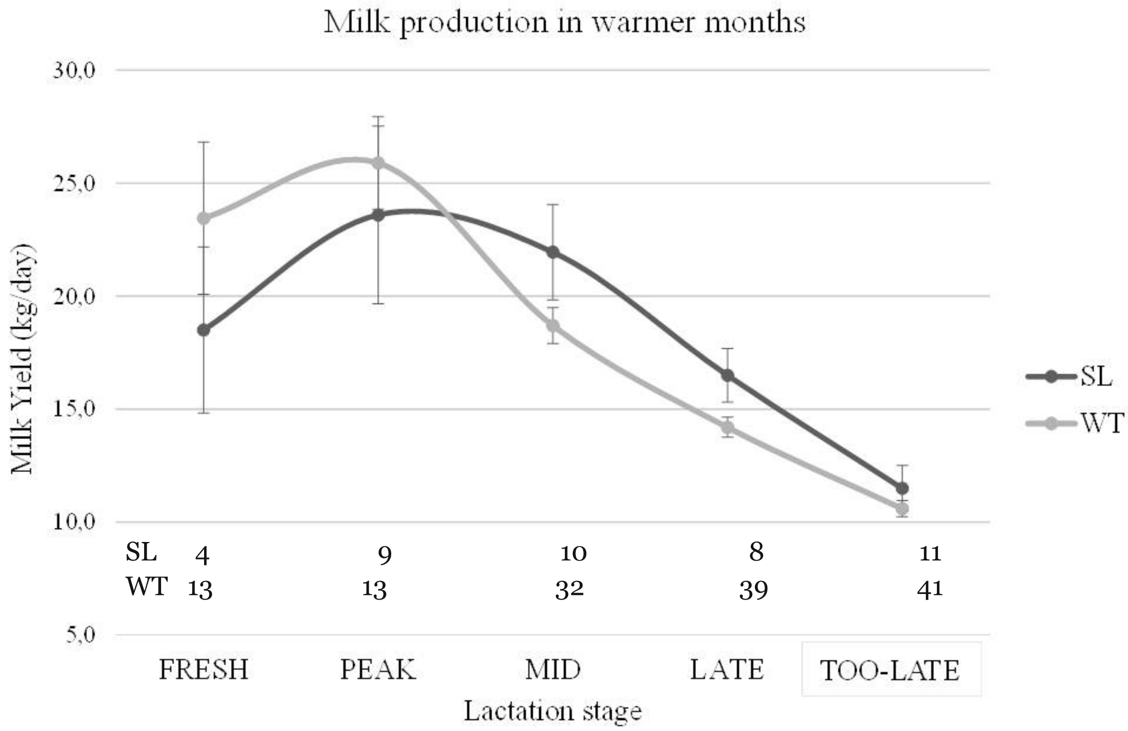

Figure 3. Lactation curve comparison between Slick and Wild-type Holsteins during hot period in Puerto Rico. Average THI ranged between 72-78. Numbers at the bottom of the graph are the number of animals in each group and stage. Lactation stage Fresh = 1-39 DIM, Peak = 40-100 DIM, Mid = 101-199 DIM, Late = 200-305 DIM, Too-late = >305 DIM. Black line = SL lactation curve; gray line = WT lactation curve.

Calving interval

From 2013-2016, the CI of SL Holsteins was 1.64 months shorter (p = 0.001) than WT Holsteins (Table 2). A herd of cows with a shorter CI would mean that on average, that herd will have less DIM at any giving moment relative to a herd of cows with longer CI, and consequently, by having less DIM, would have a greater average milk yield. Heat stress is known to affect reproductive efficiency in cattle. Previous studies document that under heat stress Holstein cattle have longer CI and days open (El-Tarabany and El-Tarabany, 2015). Also, heat stress has been associated with shorter and un-detectable estrous, leading to longer CI (Younas *et al.*, 1993, Rensis and Scaramuzzi, 2003). A THI above 72 has been associated with a decreased in conception rates and first service pregnancy rates (McGown *et al.*, 1996; Morton *et al.*, 2007; Dash *et al.*, 2016). Furthermore, cows calved during hot months have shown longer number of services (Amit and Ghandi, 2011).

It is reasonable to state that the SL phenotype in dairy cattle could confer a reproductive advantage by better coping with heat stress. The negative effects of heat stress on reproduction begin in the early developmental stages. Hansen (2019) stated that embryo quality could be affected by changes in patterns of folliculogenesis during heat stress. Additionally, oocytes and embryos are susceptible to heat stress and hot environmental conditions lead to decreased embryonic survival (Bertipaglia *et al.*, 2018). Perhaps, even under heat stress, SL oocytes are of better quality, resulting in a greater probability of fertilization and improvements in embryo development that result in a positive pregnancy and thus in a shorter calving interval. It has been shown that embryo viability at day 7 and pregnancy rate decrease during the hot period in comparison with cooler seasons (Ryan *et al.*,1993; Bertipaglia *et al.*, 2018). Thus, having an animal with superior heat tolerance and reproductive traits would allow us to counteract these inadequacies. Previous researchers have suggested that the oocyte competence, embryonic growth, gonadotropin secretion, ovarian follicular growth, steroidogenesis, development of the corpus luteum, and uterine endometrial responses are all negatively hindered when bovines are under heat stress (Hansen, 2019; Wolfenson and Roth, 2019). Thus, we propose that the SL reproductive advantage over the WT counterpart under heat stress is a result of better-quality oocytes, balanced hormonal secretions and detectable estrous that will lead to greater pregnancy rates and these parameters will be analyzed in future studies.

Table 2. Comparison of Calving Interval between WT Holsteins and SL hair Holsteins in El Remanso Dairy Farm dairy farm in Puerto Rico.

Year	SL	WT	P-value
2013	13.58 ± 0.22	15.70 ± 0.59	0.0005
2014	13.89 ± 0.23	15.74 ± 0.30	<0.0001
2015	14.05 ± 0.37	16.08 ± 0.34	<0.0001
2016	15.57 ± 0.36	16.44 ± 0.40	<0.0001

Although, no significant differences in milk production between SL and WT Holsteins were found, the SL hair genotype still represents a favorable alternative for dairy farmers in tropical regions because of their superior thermotolerance (Ríos-Solís *et al.*, 2019a) and the superior reproductive performance documented in this study. The registered SL Holsteins in Puerto Rico are the product of cross breeding with Criollo animals that began 60 years ago along with additional selection for desired qualities (Molina-Fernández, 2001). Further genetic selection could result in improved milk yields; consigning the SL gene and other uncharacterized Criollo genes as important tools to improve milk efficiency in tropical dairy systems. Additional studies that focus on the reproductive and thermoregulatory advantages of SL Holsteins and Criollo cattle are needed to better characterize the potential beneficial role of the Criollo gene pool in tropical dairy farming.

Conclusions

In the present study, no differences in milk production were found between SL and WT Holstein. Regarding the interval between calving's, the SL Holstein had an advantaged of 1.64 months compared to WT Holsteins. The research team proposed that the putative milk production advantage of SL cows documented in dairy farms in Puerto Rico is the result of a shorter CI, therefore lower DIM, and greater average milk yield as a consequence. Because under heat stress SL Holstein lactating cows seem to perform reproductively better than WT Holsteins, the SL gene may be an appropriate adaptive strategy to support an efficient dairy industry within a warmer global climate. The reproductive advantage of SL Holsteins demonstrates the potential importance of Criollo cattle in helping the dairy industry across the globe better adapt to climate change. Further studies assessing the reproductive advantages utilizing other reproductive parameters are need for a better understanding of the SL phenotype potential.

Animal use and care. This was an observational study and data collection did no required interactions with animals since the data was collected from the Dairy Records Management System. Additionally, El Remanso Dairy Farm production follows the guidelines from the University of Puerto Rico, Mayagüez Campus Institutional Animal Care and Use Committee.

Conflict of interests. The authors disclose no conflict of interest.

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Appendix A- Descriptive data

Table 3. Descriptive data for the variables analyzed during the study.

GEN	Stage of lactation	Lactation number	n	Average milk yield (kg/d)	S.D.
SL	TOOLATE	1	5	30.2	9.91
SL	TOOLATE	2	7	31.14	11.11
SL	TOOLATE	3	11	20.27	3.85
SL	FRESH	2	1	9.00	0
SL	FRESH	3	3	53	26.51
SL	LATE	1	2	40.5	2.12
SL	LATE	2	7	36.57	19.97
SL	LATE	3	15	35	9.19
SL	MID	2	4	50	7.53
SL	MID	3	11	46.55	14.48
SL	PEAK	2	3	63.33	5.51
SL	PEAK	3	6	44.17	26.82
WT	TOOLATE	1	60	24.93	9.23
WT	TOOLATE	2	28	23.29	7.02
WT	TOOLATE	3	25	21.68	6.18
WT	FRESH	1	4	32.5	6.03
WT	FRESH	2	6	68.5	20.23
WT	FRESH	3	4	65.25	20.9
WT	LATE	1	67	30.46	7.75
WT	LATE	2	33	34.3	12.35
WT	LATE	3	9	34.89	6.47
WT	MID	1	10	33	6.02
WT	MID	2	38	43.89	10.26