Developmental and reproductive performance differences of the slick-hair Holstein[1,2]

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ABSTRACT

Heat stress causes detrimental effects on animals, such as dairy cows and heifers, resulting in decreased production and changes in physiological aspects. Some Holsteins carry a mutation in the prolactin receptor (Slick; SL) that causes them to have shorter hair and greater heat tolerance compared with the wild-type (WT) cattle. Slick animals have lower temperatures, greater milk production and shorter calving intervals. The objectives of the current study were to describe monthly weight, reproductive performance, and mortality rates of the SL Holstein compared with the WT. Experiments were conducted at the Agricultural Experiment Substation in Gurabo, Puerto Rico, to determine average monthly weights of 88 heifers (40 SL and 48 WT) born between 2017 and 2020. Heifers' estrus (n=22 SL; n=27 WT) was synchronized followed by timed artificial insemination to determine differences in pregnancy rates. A subset of lactating cows was used to compare number of services required for pregnancy. Mortality frequency between genotypes was analyzed and compared. Data were analyzed using SAS University Edition, 2018 Proc-GLIMMIX and Tukey test. Compared to WT heifers, SL heifers were heavier after 14 months, required one to two fewer insemination services and died at a lower frequency rate (22% vs. 78%). Genetically selecting heat tolerant SL cows over WT may improve reproductive performance.

Key words: slick Holstein, heat stress, weight gain, pregnancy rate, mortality rate

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RESUMEN

Diferencias en el desarrollo y desempeño reproductivo de vacas Holstein pelonas

El estrés calórico tiene efectos detrimentales en los animales, como el ganado lechero, que resultan en menor producción y cambios en aspectos fisiológicos. Algunos ejemplares de vacas Holstein portan una mutación en el receptor de prolactina (pelona; P) que causa que tengan el pelo corto y les confiere mayor tolerancia al calor comparado con ganado tipo silvestre (no pelona; NP). Se ha encontrado que los animales P tienen menor temperatura, mayor producción de leche, e intervalos entre partos más cortos. Los objetivos del presente estudio eran describir los pesos mensuales, desempeño reproductivo, y tasa de mortalidad de la Holstein P comparada con la NP. Se llevaron a cabo experimentos en la Subestación Experimental Agrícola de Gurabo, Puerto Rico, para determinar los pesos promedio de 88 novillas (40 P y 48 NP) nacidas entre 2017 y 2020. El estro de las novillas (n=22 P; n=27 NP) fue sincronizado seguido de inseminación artificial en tiempo fijo para determinar las diferencias en la tasa de preñez. Un subgrupo de vacas lactando se utilizó para comparar el número de servicios requeridos para la preñez. La frecuencia en mortalidad fue analizada y comparada entre genotipos. Los datos fueron analizados usando SAS (Edición Universitaria, 2018) Proc-GLIMMIX y prueba Tukey. En general, las novillas P pesaron más luego de los 14 meses, requirieron de uno a dos servicios de inseminación menos y murieron con menor frecuencia (22% vs. 78%). Seleccionar genéticamente a las vacas P tolerantes al calor es una posible alternativa para mejorar la reproducción.

Palabras clave: Holstein pelona, estrés calórico, ganancia en peso, tasa de preñez, tasa de mortalidad

INTRODUCTION

The dairy industry in the tropics faces substantial economic losses due to elevated temperatures and humidity (St-Pierre et al., 2003). In cows, the resulting heat stress leads to decreased dry matter intake, lower milk production and impaired fertility (de Rensis and Scaramuzzi, 2003). Economic losses of U.S. livestock industries due to heat stress have been estimated at approximately $1.9 billion annually (St-Pierre et al., 2003) and continue to mount with climate variability, increased temperature averages and production costs (feed, electricity, water, labor, etc.). Dairy farming in Puerto Rico accounts for nearly 25% of total agricultural income (Progressive Dairy, 2012; Scientia, 2020). Due to warm and humid weather, however, farmers struggle to maintain their businesses. Heat abatement strategies have been implemented to mitigate losses related to heat stress (Negrón-Pérez et al., 2019), yet decreased production outputs are still evident (Hansen et al., 2004). Therefore, developing an animal with improved thermotolerance through genetic selection would represent a more efficient option to reduce losses related to heat stress.

A negative correlation between heat stress and fertility was described in the early 2000s (de Rensis and Scaramuzzi, 2003; St-Pierre et al., 2003). Moreover, during summer months fertility rates can decrease up to 10% (Hansen and Fuquay, 2011), when more animals are categorized as reproductively inefficient. Reproductive events that are affected by heat stress include: decreased estrous behavior and detection, altered ovarian function (i.e., follicular development and hormone production), and increased embryonic mortality (Collier et al., 1982; Putney et al., 1988; Wolfenson et al., 2000; de Rensis and Scaramuzzi, 2003; Habeeb et al., 2018). Negative residual effects can last for months after exposure to heat stress (Wolfenson et al., 2000).

In Caribbean cattle, researchers identified a dominant gene at the SLICK locus (Olson et al., 2003; Mariasegaram et al., 2007). Animals that carry this gene have shorter hair, fewer follicles, and larger sweat glands, which allow them to better regulate their body temperature and adapt to tropical weather (Landaeta-Hernández et al., 2011). This gene was discovered in Senepol, Carora, and Romosinuano breeds (Olson et al., 2003) and, in Puerto Rico, naturally introduced into Holstein cattle by crossbreeding Holstein cattle with Creole or Criollo animals (Sánchez-Rodríguez, 2019). Further research demonstrated that slick (SL) haired Holsteins have lower body and vaginal temperatures and increased sweating rates when exposed to heat stress (Dikmen et al., 2008, 2014). This superior thermoregulation ability prompted increased milk production when compared with their contemporary wild type lactating cows (Dikmen et al., 2014; Ortiz-Uriarte et al., 2020). Although body temperature regulation and milk production have been well documented in slick animals, reproductive variables of slick haired Holstein have yet to be extensively explored.

Decades-old observational studies in Puerto Rico suggest that the inclusion of SL Holsteins in the herd may be advantageous as they are less affected by tropical climate. Olson et al. (2003) reported that there was an indication that SL haired calves' growth rates were potentially higher than those of wild type (WT) ones in warmer months but not different in cooler months. Results from another study conducted on select farms in Puerto Rico concurred that SL haired cows had greater milk yield and shorter calving intervals (Jiménez-Cabán et al., 2015; Patiño Chaparro, 2016; Ortiz-Uriarte et al., 2020). Nonetheless, heifer conception rates or number of insemination services between SL and WT Holsteins cows have not been reported. Based on the information available, we hypothesized that, when exposed to heat stress, SL heifers would perform better than their WT contemporaries. To test our hypothesis, data from the University of Puerto Rico – Mayagüez dairy herd was analyzed to compare heifer growth rates, heifer conception rates and pregnancy age, cow number of services to conception, and heifer mortality rates between genotypes. The temperature humidity index (THI) was used to measure heat stress levels throughout the years.

MATERIALS AND METHODS

Farm location and herd information

Animal use was approved by the University of Puerto Rico, Mayagüez Campus (UPRM), *Institutional Animal Care and Use Committee*. Studies were conducted at the UPRM Agricultural Experiment Substation in Gurabo, Puerto Rico (18°15'07.6"N 65°59'25.5"W). Our observations of the milking cows were recorded at the UPRM dairy farm located in Lajas, Puerto Rico (18°01'30.8"N 67°04'33.3"W).

The number of animals used for each study varied depending on the trait observed, animal age and genotype. All animals considered in this study were previously genotyped using an in-house TaqMan Real Time PCR assay (Sosa et al., 2021). The average heifer herd size in Gurabo is approximately 150 head composed of seven age groups (Table 1). Fifty percent of the herd is of known slick (SL) or wild type (WT) genotype, of which 35% are SL and 65% are WT.

All animals were on a semi-intensive rotational grazing system based on *Megathyrsus maximus* and *Cynodon nlemfuensis*, and supplemented twice a day (7:00 and 13:00) with 2.25 kg of a commercial dairy concentrate (Heifer Ration, 16% crude protein, "*Federación de Asociaciones Pecuarias de Puerto Rico, Inc.*"[5]) offered on feed bunks located in the pasture. Animal weight gain was recorded once a

TABLE 1.—*Heifer numbers in Gurabo, Puerto Rico, by age.*

Age range (months)	Average herd size
2-6	4
6-8	11
8-12	37
12-16	11
16-20	12
20-24	15
24-36	60

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[5] Company or trade names in this publication are used only to provide specific information. Mention of a company or trade name does not constitute an endorsement by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

month. Heat stress exposure was determined by calculating monthly minimum, average, and maximum temperature humidity indexes of years 2017 to 2020 (Figure 1). Temperature humidity index was calculated using the formula: THI=(1.8*AT+32) - [(0.55-0.0055*RH) x (1.8*AT-26)], where AT=air temperature (°C), and RH=relative humidity (%) (Habeeb et al., 2018). Air temperature and relative humidity were obtained from meteorological stations (HOBOlink) located in Gurabo and Lajas. From August 2017 to July 2018 accurate meteorological data was not available. Thus, temperature and humidity data were obtained either from National Weather Service NOAA Online Weather Data (National Oceanic and Atmospheric Administration, 2004) or estimated based on the data from 2019 and 2020. The HOBOLink meteorological station recorded climatological conditions every 30 minutes. This is representative data of two ecological zones, subtropical moist forest (central east, Gurabo) and subtropical dry forest (southwest, Lajas), of Puerto Rico. Based on average and maximum THI values depicted in Figure 1, and according to Zimbelman

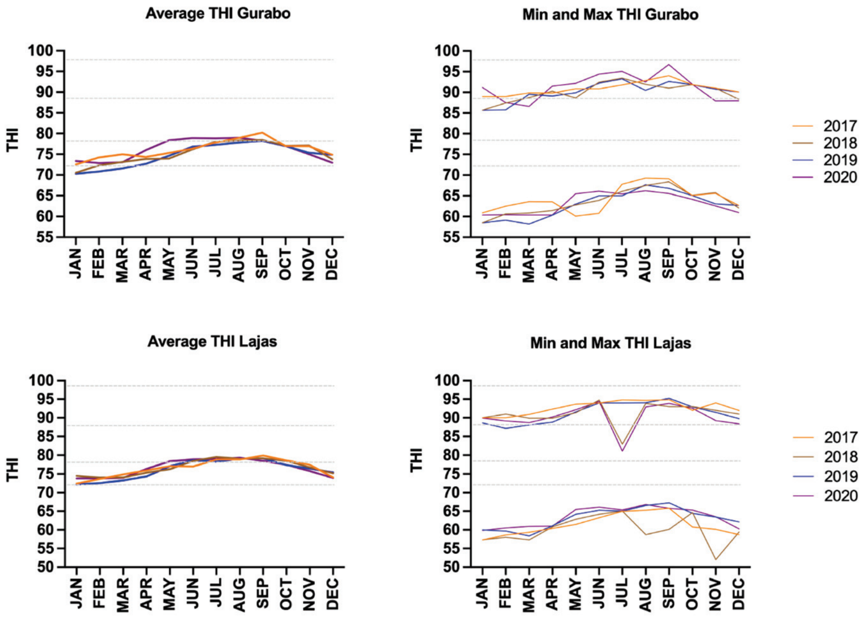

FIGURE 1. Average temperature humidity indexes (THI) at Gurabo and Lajas, Puerto Rico, during 2017-2020. Values were recorded every 30 minutes throughout 2017-2020 (HOBOlink meteorological stations). Colored lines indicate monthly average THI values (arbitrary units) for each year (orange = 2017, brown = 2018, blue = 2019 and purple = 2020). Hyphenated horizontal lines indicate thresholds: mild (72 to 78), moderate (79 to 88) and severe (89 to 98), when cows begin to experience heat stress.

et al. (2009), animals in Puerto Rico experience moderate (79 to 88) to severe (89 to 98) heat stress throughout the year during the day, and for at least 12 hours of the day, respectively. During the remaining time, dairy animals are exposed to either none or mild (72 to 78) heat stress (Figure 2).

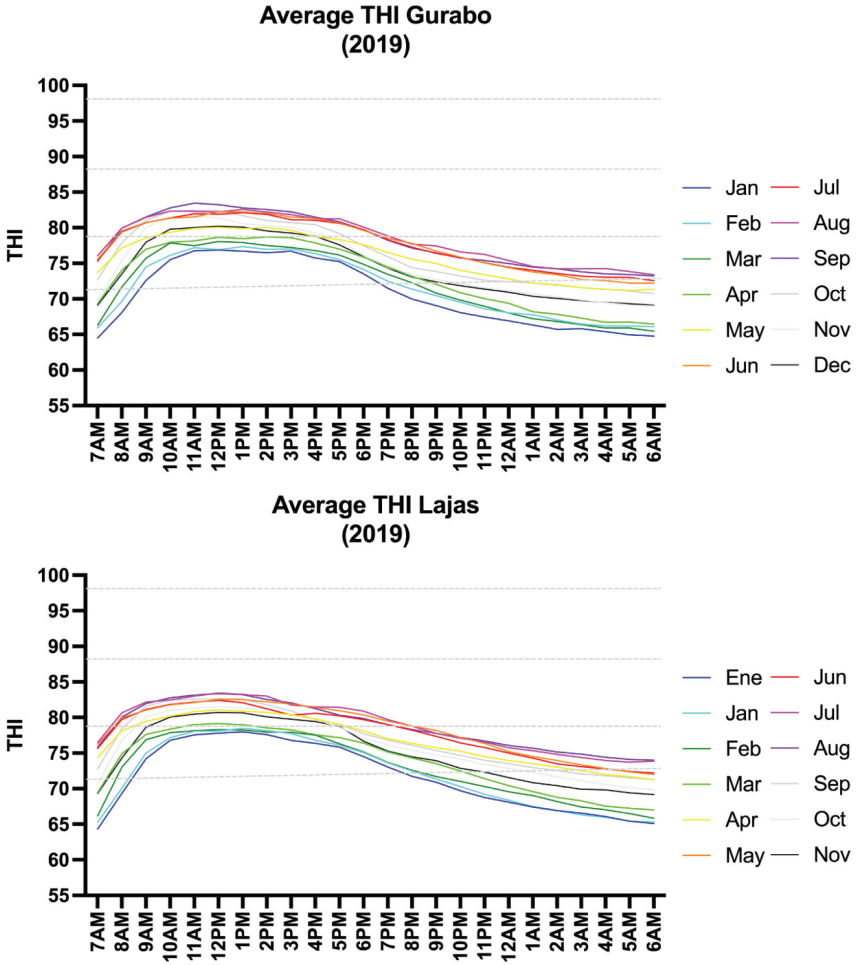

FIGURE 2. Average temperature humidity indexes (THI) per hour at Gurabo and Lajas, Puerto Rico during 2019. Values were recorded every 30 minutes throughout 2019 (HOBOlink meteorological stations). Colored lines indicate monthly average THI values (arbitrary units) for each hour of the day. Hyphenated horizontal lines indicate thresholds: none (0 to 71), mild (72 to 78), moderate (79 to 88) and severe (89 to 98), when cows experience heat stress.

Estrous synchronization

A group of 49 heifers (SL=22 and WT=27) were subjected to Select Sires, Inc. 5-day CoSynch synchronization protocol from October 2019 to June 2020. Heifer age and weight ranges at first breeding were 18 to 24 months and 285 to 500 kg for the SL group, and 21 to 36 months, and 270 to 525 kg for the WT group. A total of six estrous synchronization and AI procedures were done over the nine months of the trial. All animals received an injection of GnRH (100 µg as 2 mL of Cystorelin®, Zoetis, i. m.) on days -8 and 0, and an injection of PGF2α (25 mg as 5 mL of Lutalyse®, Zoetis, i.m.) on days -3 and -2. Timed artificial insemination (TAI) was done in the morning of day 0. Pregnancy success was evaluated by an experienced veterinarian at day 60 post TAI. Non-pregnant heifers were re-synchronized and serviced a second time. For the third time, non-pregnant heifers were grouped with a bull for natural service.

Data on lactating cows was obtained from the Dairy Records Management System, Dairy Herd Improvement Association (DHIA) records (DRMS; www.drms.org). Cows included in this study last calved between the months of July 2020 and March 2021 and were inseminated from September 2020 through May 2021. Protocols for estrous synchronization were the same as previously described. Non-pregnant cows were artificially inseminated repeatedly until pregnant.

Statistical Analysis

Data were analyzed using SAS software (SAS University Edition, 2018); each individual animal was considered an experimental unit. Separate models were used to analyze the data from each location. Additionally, separate models were used to analyze variables for heifers and cows. Differences in monthly weight, breeding age, breeding weight, pregnancy age, pregnancy weight and number of services for pregnancy by genotype were determined by using Proc GLIMMIX and the Tukey-Kramer adjustment. All models for monthly weight, breeding age, breeding weight, pregnancy age, pregnancy weight and number of services to pregnancy included genotype as a fixed effect. Cow, location and calendar year were included as random effects. Other variables evaluated and included in the models as fixed are: birth month, birth season, THI at birth, transport month, transport season and THI at transport. Animal transport occurred after weaning the animals at three months of age. Based on Puerto Rico's location in the hemisphere, changes in temperature, weather and number of daylight hours fluctuate to a minimum; thus, the season was defined as warm (April to September) or cool (October to March). Additionally, THI was categorized as none (0 to 71), mild (72 to 78), moderate (79 to 88) and severe (89 to 98). Because of the limited number of animals, we were unable to account for heifer sires, heifer dams, and dam parity in our analysis of body weight gain. Levels of main effects were separated using the PDIFF option. Results for fixed effects are presented as least-squared means ± standard error of the means. Proportion of heifer breeding success and mortality rates by genotype were calculated using the frequency procedure (Proc FREQ). Results are presented as a percentage of the total for each genotype. Statistical differences were reported when variation was $P < 0.05$; tendencies were considered when $0.10 > P > 0.06$.

RESULTS

Monthly weight gain

A total of 88 heifers (SL n=40; WT n=48) were weighed for this analysis. Birth weights and weights up to three months of age were obtained from the dairy birth records at Lajas where they stayed until weaned; growth weights (three months onwards) were obtained from records at Gurabo where they were raised. Animals at a given age were chosen at random to be weighed and thus, the number of observations for a given animal varies throughout the study. Figure 3 depicts a summary of the growth curve for each genotype from 0 to 36 months of age; Figure 4 depicts all data points used for this study. Table 2 includes the *n* values and average numbers for each group by age. Although numerically SL heifers weighed more (approx. 5 to 20 kg) over the entire weighing period, statistical differences among the groups were significant after 14 months old age. At an early age (0 to 13 months), both the SL and WT heifers were approximately the same weight ($P > 0.13$). However, at 14 months onwards ($P \leq 0.04$), the SL heifers were on average 30 to 45 kg heavier than the WT.

When including season of birth, THI at birth, season of transport, and THI at transport in the model, there were significant effects found from ages 4 to 8 and 14 to 28 months old in addition to the genotype effect. Heifers born and/or transported in the warmer months weighed less than those born and/or transported in the cooler months ($P \leq 0.03$). Similarly, heifers born and/or transported in moderate THI weighed less than those born and/or transported under mild THI conditions ($P \leq 0.02$). Month of birth and month of transport had no effect on weight gain at any age.

Pregnancy rates

Based on the growth data discussed in the previous section, slick heifers reached breeding weight (i.e., 317.5 to 362.8 kg; approx. 75% of

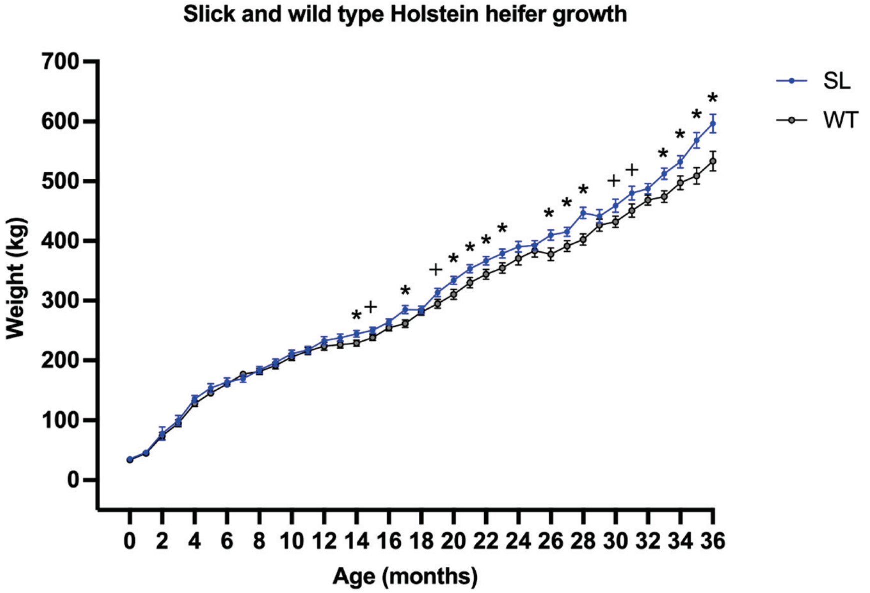

FIGURE 3. Monthly weight gain by genotype. Weight averages (kg) of 88 animals (N = 40 SL, N = 48 WT; different *n* subsets depending on the age) at specific age (months) were analyzed to compare differences between SL and WT Holstein heifers. Asterisks (*) above data points indicate significant differences ($P < 0.04$). Plus symbols (+) above data points indicate a tendency to differ ($0.10 > P > 0.07$).

the estimated mature weight) two months earlier than their WT counterparts. To further evaluate their fertility based on breeding success, a group of heifers were synchronized and artificially inseminated (AI). Results showed that on average, SL heifers became pregnant at least five months earlier than the WT (Table 3). On average, two breeding cycles were required for both groups (SL=2.0±0.18 vs. WT=1.89±0.16; P=0.65) to become pregnant regardless of age and weight. Age differences were more noticeable on the third breeding when SL heifers became pregnant at 24 months of age and WT heifers were nearly 36 months old (P = 0.003).

A similar observation in terms of pregnancy success was found when following 14 of these heifers (7 SL, 7 WT) to their first lactation and second pregnancy (Table 4). On average, SL cows in their first lactation became pregnant at 37 months and required 1.3 AI services compared with the WT, which became pregnant at 48 months and required 2.6 AI services (P = 0.02). Results were not significantly different for cows past the second lactation. After this point, both groups required an average of 2.9 AI services to become pregnant.

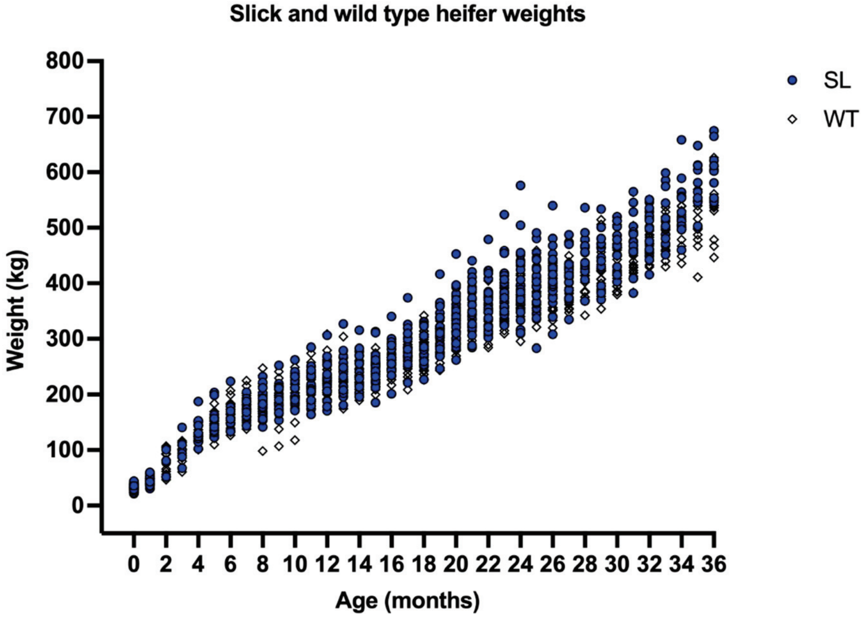

FIGURE 4. Holstein heifer weights by genotype over time. A total of 88 animals (N = 40 SL, N = 48 WT) of various age groups (months) were subjected to analysis to compare monthly weight averages (kg) between SL (circles) and WT (diamonds) Holstein heifers.

Mortality rates

Mortality rates were recorded after natural death causes (digestive or metabolic disorders, respiratory problems, and unknown reasons) for heifers in the herd for years 2019 (14/150) and 2020 (20/150). Of the 34 animals that died during both years (Table 5), only 23 were of known genotype. Out of those 23, five (21.7%) were SL and 18 (78.3%) were WT. The same difference in frequency of death was recorded in both years. In terms of age of death there was a tendency (P = 0.07) but not a significant difference between genotypes (SL = 16.5 months vs. WT = 25.6 months).

DISCUSSION

Heat stress has a negative impact on dry matter intake (West, 1999), nutritional status (Nonaka et al., 2008), reproduction (de Rensis and Scaramuzzi, 2003; St-Pierre et al., 2003; Negrón-Pérez et al., 2019) and overall health (Wang et al., 2020). Earlier studies showed that the SL Holstein has better thermoregulation ability

Table 2.—*Slick and wild type average weights (kg) by age (months).*

	Slick (N = 40)			Wild type (N = 48)			Adjusted LS Means[1]		
Age	*n*	Weight	S.E.	*n*	Weight	S.E.	Differences	S.E.	P-value
0	40	35.33	0.76	48	33.75	0.70	1.57	1.04	0.132
1	17	46.36	1.62	20	44.75	1.49	1.61	2.20	0.469
2	4	77.93	10.93	13	73.95	6.06	3.97	12.50	0.755
3	6	99.99	8.33	12	95.16	5.89	4.83	10.21	0.643
4	10	135.68	5.93	16	128.08	4.69	7.60	7.56	0.325
5	9	154.06	7.07	25	145.57	4.24	8.49	8.24	0.311
6	10	163.79	6.80	29	160.71	3.99	3.08	7.89	0.699
7	13	169.56	5.82	27	176.83	4.04	-7.27	7.08	0.311
8	21	183.83	5.80	25	181.65	5.32	2.18	7.87	0.783
9	21	196.34	5.76	24	191.40	5.39	4.94	7.89	0.534
10	22	211.08	5.97	23	205.96	5.84	5.12	8.36	0.544
11	22	217.61	6.09	23	215.49	5.96	2.12	8.52	0.805
12	22	233.14	6.72	23	223.68	6.57	9.46	9.39	0.320
13	24	237.85	6.20	25	226.41	6.07	11.44	8.68	0.194
14	23	244.56a[2]	5.63	26	228.94b	5.30	15.62	7.74	0.049
15	22	250.22[+]	5.23	25	238.13	4.91	12.09	7.17	0.099
16	22	264.29	5.35	24	254.79	5.12	9.50	7.41	0.207
17	20	285.32a	6.71	22	261.61b	6.40	23.72	9.27	0.014
18	20	284.78	6.27	29	280.92	5.21	3.86	8.15	0.638
19	25	313.78[+]	7.02	22	294.72	7.48	19.06	10.26	0.070
20	29	334.18a	6.82	19	310.39b	8.43	23.79	10.84	0.033
21	27	353.57a	6.84	17	330.15b	8.62	23.42	11.01	0.039

[1] Data is represented as least squared means ± S.E.
Adjusted L.S. Means differences ± S.E. are weight difference between genotypes (slick – wild type).

[2] Variables with different letters are significantly different from their counterpart. Statistical comparisons are between columns (slick *vs.* wild type).

[+] Statistical tendency to differ (0.10 > P > 0.07).

TABLE 2.—*(Continued) Slick and wild type average weights (kg) by age (months).*

	Slick (N = 40)			Wild type (N = 48)			Adjusted LS Means[1]		
Age	n	Weight	S.E.	n	Weight	S.E.	Differences	S.E.	P-value
22	26	366.87a	7.23	19	343.97b	8.45	22.90	11.12	0.046
23	26	378.77a	7.73	20	354.80b	8.82	23.97	11.73	0.047
24	29	390.18	9.17	20	370.75	11.05	19.44	14.36	0.182
25	28	392.50	7.97	16	383.48	10.54	9.02	13.21	0.499
26	26	409.74a	8.49	16	377.67b	10.82	32.08	13.75	0.025
27	22	415.40a	7.41	15	391.61b	8.97	23.79	11.64	0.049
28	16	447.07a	9.33	16	402.51b	9.64	44.57	13.42	0.002
29	16	441.61	11.13	19	426.78	10.22	14.84	15.11	0.333
30	14	459.01^{+}	10.89	19	431.91	9.61	27.09	14.52	0.072
31	16	480.04^{+}	11.33	18	450.74	10.99	29.29	15.78	0.073
32	18	487.76	8.73	20	468.27	8.50	19.48	12.18	0.119
33	16	512.45a	9.70	15	474.39b	10.01	38.07	13.94	0.011
34	16	532.39a	10.29	13	497.45b	11.42	34.94	15.37	0.031
35	12	568.58a	13.17	11	509.03b	13.76	59.55	19.04	0.005
36	11	596.58a	15.56	10	533.71b	16.32	62.86	22.55	0.012

[1] Data is represented as least squared means ± S.E.
Adjusted L.S. Means differences ± S.E. are weight difference between genotypes (slick – wild type).

[2] Variables with different letters are significantly different from their counterpart. Statistical comparisons are between columns (slick *vs.* wild type).

$^{+}$Statistical tendency to differ ($0.10 > P > 0.07$).

TABLE 3.—*Comparison of pregnancy age, weight and breeding success of Holstein slick (N=22) and wild type (N=27) heifers.*

	Slick	Wild type	*P*-value
	N=22	N=27	—
Breeding age[1]	22.2 ± 1.1 a[2]	27.3 ± 0.9 b	0.001
Breeding weight	367.8 ± 13.1	381.9 ± 11.8	0.427
Pregnancy age	25.2 ± 1.4 a	30.4 ± 1.2 b	0.007
Pregnancy weight	415.9 ± 16.3	420.1 ± 14.7	0.850
1st Breeding attempt success	36% (8/22)	37% (10/27)	—
Breeding age	24.0 ± 1.1	24.6 ± 1.0	0.679
Breeding weight	374.8 ± 19.9	359.7 ± 17.8	0.579
Pregnancy age	24.0 ± 1.1	24.6 ± 1.0	0.679
Pregnancy weight	374.8 ± 19.9	359.7 ± 17.8	0.579
2nd Breeding attempt success	43% (6/14)	59% (10/17)	—
Breeding age	22.5 ± 2.1 a	28.7 ± 1.6 b	0.035
Breeding weight	388.3 ± 24.4	394.6 ± 18.9	0.841
Pregnancy age	28.3 ± 2.1	31.3 ± 1.6	0.271
Pregnancy weight	459.3 ± 27.3	429.3 ± 21.3	0.404
3rd Breeding attempt success	100% (8/8)	100% (7/7)	—
Breeding age	20.3 ± 2.1 a	29.3 ± 2.2 b	0.010
Breeding weight	345.3 ± 24.4	395.5 ± 26.1	0.184
Pregnancy age	24.1 ± 2.5 a	37.3 ± 2.7 b	0.003
Pregnancy weight	424.5 ± 24.1[+]	493.2 ± 25.8	0.074

[1] Breeding age (months) and weight (kg) represent when the heifer was first bred regardless of whether she became pregnant or not. Pregnancy age and weight represent when heifer became pregnant after the first, second or third attempt, respectively. The initial rows are an average of all the animals together regardless of when they became pregnant. Data is represented as means ± S.E.M.

[2] Variables with different letters are significantly different from their counterpart. Statistical comparisons are between columns (slick vs. wild type).

[+] Statistical tendency to differ (0.10 > P > 0.07).

and seems to be less affected by heat stress when compared with WT type animals (Dikmen et al., 2008; Landaeta-Hernández et al., 2011). Results reported here suggest that when measuring weight

TABLE 4.—*Reproductive performance of SL Holstein cows compared with WT.*

	Slick	Wild type	*P*-value
1st lactation (n)	7	7	—
Age at 2nd pregnancy[1]	37.4 ± 1.4 a[2]	47.7 ± 1.4 b	0.002
Weight	564.3 ± 17.7	570.2 ± 17.7	0.818
Number of services for pregnancy	1.3 ± 0.3 a	2.6 ± 0.3 b	0.017

[1] Age (months) and weight (kg) values indicate when the cow was confirmed as pregnant for the second time. Data is represented as means ± S.E.M.

[2] Variables with different letters are significantly different from their counterpart. Statistical comparisons are between columns (slick vs. wild type).

TABLE 5.—*Heifer mortality rates in Gurabo, Puerto Rico, for years 2019 and 2020.*

Year	Total (genotyped)	Slick	Wild type
2019	9.3% 14/150 (9/14)	22.2% (2/9)	77.8% (7/9)
2020	13.3% 20/150 (14/20) —	21.4% (3/14)	78.6% (11/14)
Total	(23/34)	21.7% (5/23)	78.3% (18/23)
Average age (months)	—	16.5 a	25.6 a

Average age (months) of death values with same letters tend to be, but are not significantly different from each other (P = 0.07).

gain, both genotypes were similar at early stages, but the SL heifers grew heavier at some point, and reached reproductive weight sooner than the WT. Further, the SL heifers became pregnant at an earlier age, and required fewer insemination services at the first lactation than the WT. This may be interpreted as the SL animals being more fertile than the WT. This is not the first time this has been suggested as the calving interval between genotypes favors the SL Holstein (Patiño-Chaparro, 2016; Ortiz-Uriarte et al., 2020). In another study, insulin and progesterone levels were compared before and during estrus synchronization, and after artificial insemination. No differences in progesterone levels were found, but insulin levels were greater in WT heifers (Marrero-Torres, 2021). Previous research has found that elevated insulin levels caused by heat stress are associated with negative effects on fertility (Rhoads et al., 2009). This may be compromising the WT heifers. Or it's possible that the SL genotype confers reproductive advantages that may be better understood with further research.

Ongoing studies are being conducted to identify if there are differences in specific genes associated with fertility between genotypes. Alternatively, other differences in the embryo development or events in the animals at an early age may have detrimental effects on the WT cattle that carry over and affect adult animal performance. To consider this, we observed and analyzed the genotype frequency in mortality rates. Present results indicate that the WT heifer is more likely to be affected by common causes of death at an older age than the SL heifer.

The SL gene was inherited from Carora, Romosinuano and Senepol breeds in the Caribbean islands (Olson et al., 2003). In the case of Puerto Rico, natural crosses to local Creole and Holstein animals conferred the SL gene; over the years, these genetics were diluted, and current animals have become almost 100% Holstein (Ortiz-Uriarte et al., 2020). It is very likely that the SL animals inherited other genes associated with the animals' immune response along with characteristics to resist diseases. An example of this is tick resistance; Creole cows have a natural resistance to ticks (de Alba and Carrera, 1958; Frisch, 1999), and cows with shorter hair have fewer ticks (Foster et al., 2008). Additionally, it is plausible that because the animal is more heat tolerant, when exposed to heat stress the SL heifer's immune response is less compromised and thus the animal is more likely to survive a disease than the WT.

These results are of great importance to producers in the tropics when considering economical investments and returns. In general, the information presented here suggests that raising WT cattle in the tropics is more expensive than raising those with the SL gene. By the time a WT heifer is pregnant or calving for the first time, a SL heifer will already be in production and close to her second pregnancy. In addition, in terms of survival, the SL heifer may represent a better return on investment as these have a lower mortality rate relative to the WT.

Animals in the current study were bred much later than conventionally practiced, thus cows were older on their first, second and future lactations. Slick heifers became pregnant when they should have been calving, if compared to conventional practices where heifers are expected to calve by 24 months. Thus, SL heifers were nine months behind the ideal production schedule. As for the WT heifers, they were 15 months behind the conventional practice schedule, becoming pregnant at 30 months of age. This is not uncommon in the tropics as animals growing under warmer climatic conditions and caloric stress reach puberty and mature body weight (65 percent of their expected weight) at a later age (Dado-Senn et al., 2021; De Rensis et al., 2021). Therefore, the current data presented here suggests a great advantage for SL heifers, especially if raised under stressful climatic conditions, as they perform better than their WT contemporaries.

In conclusion, the data supported the hypothesis that, in tropical regions, incorporating the SL gene in cattle can potentially increase producers' profits by improving overall herd production, reproduction, performance and health. Due to the limitation of sample numbers, this analysis needs to be broadened. To further identify specific components involved in the superior performance and the potential advantages of SL vs. WT cows, perhaps molecular analysis, in depth studies of the animal records and larger data sets need to be considered.

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