The Larson Blue coat color phenotype in Holsteins: Characteristics and effects on body temperature regulation and production in lactating cows in a hot climate[1]

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ABSTRACT: Here we report a previously undescribed coat color phenotype in Holstein cattle. Larson Blue Holsteins, located on a dairy in south Florida, exhibit a coloration pattern that is similar to that of black and white or red and white Holsteins except that, instead of being black or red, darker regions of the body vary in color from gray to taupe. The Larson Blue phenotype was readily apparent in young calves. The phenotype is not due to inheritance of known mutations causing coat color variation in cattle, including dominant red, Telstar, silver color dilutor, or Dun color. Three variants with moderate effects on the *melanophilin* (*MLPH*) gene were identified in 2 Larson blue cows. Despite being lighter in color, there was no difference in daily variation in vaginal temperature between Larson Blue and other Holsteins when recorded during the summer for cows housed in free-stall barns with shade, fans, and sprinklers. Similarly, there was no effect of the Larson Blue phenotype on seasonal variation in milk yield. Therefore, the phenotype confers no advantage in terms of response to heat stress when cattle are housed in facilities with extensive cooling.

Key words: body temperature, cattle, coat color, heat stress, milk yield

INTRODUCTION

In cattle, as for other mammals, coat color is determined largely by genes controlling synthesis of phaeomelanin (red and yellow) and eumelanin (black) pigments by melanocytes and by distribution of pigment to the skin and hair (Cieslak et al., 2011). Mutations in *v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog* (*KIT*) and *microphthalmia-associated transcription factor* (*MITF*) have been associated with the percent of the coat that is pigmented in Holsteins (Hayes et al., 2010). A major gene responsible for coat color variation is *melanocortin 1 receptor* (*MCR1*), encodes which contains alleles existing in multiple breeds that are termed $MC1R^D$ (black; dominant), $MC1R^{BR}$ (wild-type; reddish-black), and $MC1R^e$ (red; recessive), that affect functional capacity of the receptor (Switonski et al., 2013). A missense mutation in the *coatomer protein complex, α subunit* (*COPA*) involved in vesicle trafficking is responsible for the dominant red coat color in Holsteins (Dorshorst et al., 2015). Other mutations can cause a dilution of pigmentation in the coat, such as *tyrosinase related protein 1* (*TYRP1*) in Dexter cattle (Berryere et al., 2003), *premelanosome protein* (*PMEL*) in Highland and Galloway cattle (Schmutz and Dreger, 2013), and *melanophilin* (*MLPH*) in Belgian Blue cattle (Li et al., 2016).

Coat color can have an important impact on thermal balance. In cattle, white hair absorbs only about 50% of the incoming solar radiation as black hair (Stewart, 1953), and consequently, heat flux at the skin is greater for dark animals (Finch et al., 1984). Holstein cows exposed to direct solar radiation that were predominately white were better at regulating body temperature and in maintaining milk yield than predominately black cows (Hansen, 1990). In another study with Holsteins (King et al., 1988), predominately white coats were associated with better reproduction in hot weather but not greater milk yield.

Whereas the predominant coat colors in Holstein are black, red, and white, other colors exist. The Larson Blue Holstein is a cow with a typical Holstein spotting pattern but where the hair displays a greyish-brown coloration. Holstein cows with the Larson Blue phenotype have existed on the Larson Dairy, Inc., in Okeechobee, FL, since purchase of cattle of that phenotype from Illinois in 1954 (Louis Larson Sr., Larson Dairy, Inc., personal communication).

The purpose of the current study was to characterize some features of the Larson Blue phenotype and to test the hypothesis that inheritance of the phenotype is associated with increased capacity for body temperature regulation and reduced depression in milk yield in summer compared with other Holsteins.

MATERIALS AND METHODS

Animals and Housing

The study was conducted at the Larson Dairy in Okeechobee, FL (27°14′ N, 80°50′ W). Larson Blue cows were identified based on phenotype. Other Holsteins, which varied in proportions of black and white, are termed wild-type for the purposes of the study. Cows were housed in sand-bedded free-stall barns equipped with sprinklers (Rain Bird Manufacturing, Glendale, CA) and fans (J&D Manufacturing Inc., Eau Claire, WI) that were programmed to become activated when dry-bulb temperature exceeded 21.1°C. Sprinklers were placed above the feed bunk and were also arranged around the fan so that water was directed into the airstream. When activated, fans and sprinklers operated continuously and sprinklers were activated for 1.5 min at 6-min intervals. Feed and water were available ad libitum for all cows at all times. Cows were milked twice daily between 0600 and 0900 h and between 1800 and 2100 h. Animal use was approved by the University of Florida Institutional Animal Care and Use Committee.

Frequency of Coat Color Alleles

A total of 10 Larson Blue cows were genotyped for known coat color genes using the Clarifide 19K low-density test (Zoetis Animal Genetics, Kalamazoo, MI) for mutations in *MC1R*, *COPA*, and *PMEL* and using the Igenity platform for a mutation in *TYRP1* (GeneSeek, Lincoln, NE).

Whole Genome Sequencing

Genomic DNA for 2 Larson Blue cows was prepared from whole blood using a QIAamp DNA Mini Kit (QIAGEN, Valencia, CA) and shipped to Cofactor Genomics (St. Louis, MO) for sequencing library construction using dual TruSeq barcode adapters. The resulting libraries were sequenced on an Illumina NextSeq 500 at the Animal Genomics and Improvement Laboratory (Beltsville, MD) according to the manufacturer's protocols (Illumina Inc., San Diego, CA) using the NextSeq 500 version 1.0 sequencing reagent kit. Pooled libraries were sequenced as 2×150 bp paired-end reads. Sequence reads for each animal were aligned to the UMD 3.1.1 (BovineGenome, 2016) assembly of the *Bos taurus* genome assembly using BWA version 0.7.15 (Li and Durbin, 2010). Resulting SAM files were processed using samtools version 1.3 and variant calls were made using the samtools mpileup pipeline (Li et al., 2009). Variants were called and their effects on annotated genes predicted using SnpEff 4.1l (Cingolani et al., 2012).

Vaginal Temperatures during Heat Stress

Daily patterns of vaginal temperature of lactating cows of the Larson Blue ($n = 14$) and wild-type phenotype ($n = 14$) were determined over a 6-d period (August 14–20, 2014) during typical hot and humid conditions of this location. Wild-type cows were chosen to match the Larson Blue cows according to parity, days in milk, and milk yield. There was no difference ($P > 0.05$) at the start of the experiment between groups in parity (1.9 [SEM 0.2] vs. 1.9 yr [SEM 0.2] for Larson Blue and wild-type, respectively; least squares means), days in milk (157.4 [SEM 18.2] vs. 157.4 d [SEM 18.2], respectively), and most recent test day milk yield (34.5 [SEM 1.6] vs. 35.0 kg [SEM 1.6], respectively).

Vaginal temperature was measured at 5-min intervals for 6 d using an iButton data logger (model 1922T; Maxim Integrated, San Jose, CA; accuracy of ±0.0625°C at 11-bit resolution) that was attached to a blank (i.e., without progesterone) controlled internal release device (CIDR; Zoetis Inc., Florham Park, NJ). Details of the procedure are described by Dikmen et al. (2014). Dry bulb temperature and relative humidity were recorded every 5 min in the barn at a height of

Figure 1. Representative examples of cows exhibiting the Larson Blue phenotype. Panels A and B represent the same cow and demonstrate how the appearance of the coat color varies with the lighting. Shown in panel C are a black and white (left) and a Larson Blue Holstein cow (right). Panel D is a photograph of a young Larson Blue heifer calf. The Larson Blue phenotype is readily apparent from birth.

3 m using HOBO-U12 data loggers (Onset Computer Corp., Bourne, MA). The temperature–humidity index (THI) was calculated according to the equation reported by Ravagnolo and Misztal (2000):

$$\text{THI} = (1.8 \times T + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)],$$

in which T is the dry bulb temperature (°C) and RH is the relative humidity (percent).

Data on vaginal temperatures were analyzed using the MIXED procedure of SAS (version 9.4; SAS Inst. Inc., Cary, NC). The model included coat type, barn of cow, day, time, barn × time, and the coat type × time interaction. Cow nested within coat type and barn was considered random and other effects were fixed. In a second analysis, effects of coat type on average peak vaginal temperature were determined by ANOVA using the GLM procedure of SAS.

Milk Yield

A total of 13,038 lactation milk yields (lactations 1–5) were recorded for 83 Larson Blue and 8,442 wild-type Holstein cows. Data were analyzed by least squares ANOVA using the GLM procedure of SAS. The model included fixed effects of coat type, cow nested within coat type, parity (first lactation vs. others), month of freshening, year of freshening, coat type × parity, and coat type × month. Cow nested within coat type was considered random and other main effects were considered fixed.

Table 1. Frequency of common coat color alleles in Larson Blue Holsteins

Platform	Trait	Gene	Chromosome	Position, bp	Result
Clarifide[1]	Red/black	MC1R	18	14,757,910	9 BB (homozygous black) + 1 AB (wild-type carrier)
Clarifide	Red/black	MC1R	18	14,757,924	10 AA (homozygous black)
Clarifide	Dominant red	COPA	3	9,479,532	10 BB (no copies dominant red)
Clarifide	Telstar	MC1R	18	14,757,740	10 BB (Telstar free)
Clarifide	Silver color dilutor	PMEL	5	57,669,912	10 AA (free)
Bovine LD v4[2]	Dun	TYRP1	8	31,711,945	10 CC (wild-type)

[1]Zoetis Animal Genetics, Kalamazoo, MI.

[2]GeneSeek, Lincoln, NE.

Table 2. Sequence variants in *MLPH* on chromosome 3 in 2 Larson Blue cows

Location, bp	Reference allele	Alternate allele	Effect	Impact	AA change	Animal 1	Animal 2
117,606,496	G	A	Missense	Moderate	Val113Met	0/1	0/1
117,606,550	A	C	Missense	Moderate	Ser131Arg	0/1	1/1
117,606,551	G	A	Missense	Moderate	Ser131Asn	0/1	0/1

RESULTS AND DISCUSSION

Characteristic Appearance of Larson Blue Cows

Representative images of animals with the Larson Blue phenotype are shown in Fig. 1. The pattern of coloration was similar to that of black and white or red and white Holsteins except that, instead of being black or red, darker regions of the body varied in color from gray to taupe. Coloration varied with the available light

Figure 2. Environmental variables during the experiment to determine effect of coat type on vaginal temperature. Data shown are the averages of the 6 d of the study.

so that animals appeared taupe when in sunlight and more grayish when not in direct sun (compare 2 images of the same cow in Fig. 1A and 1B). The Larson Blue phenotype was readily apparent in young calves (Fig. 1D). Although the Larson Blue phenotype is prevalent in the Larson Dairy in Okeechobee, FL, a Holstein cow exhibiting similar coloration was observed in a dairy in Poland and a separate animal was observed in California (Supplementary Fig. S1). Therefore, either the genotype responsible for the phenotype is distributed beyond the herd in which it was originally described or other mutations causing a similar phenotype exist in Holstein cattle.

Larson Blue Phenotype and Inheritance of Known Coat Color Alleles

A total of 10 Larson Blue animals were tested for inheritance of alleles that are associated with color variation in cattle (Table 1). None of the cattle were positive for dominant red, Telstar, silver color dilutor, or Dun color. Of the 2 loci in *MC1R* associated with
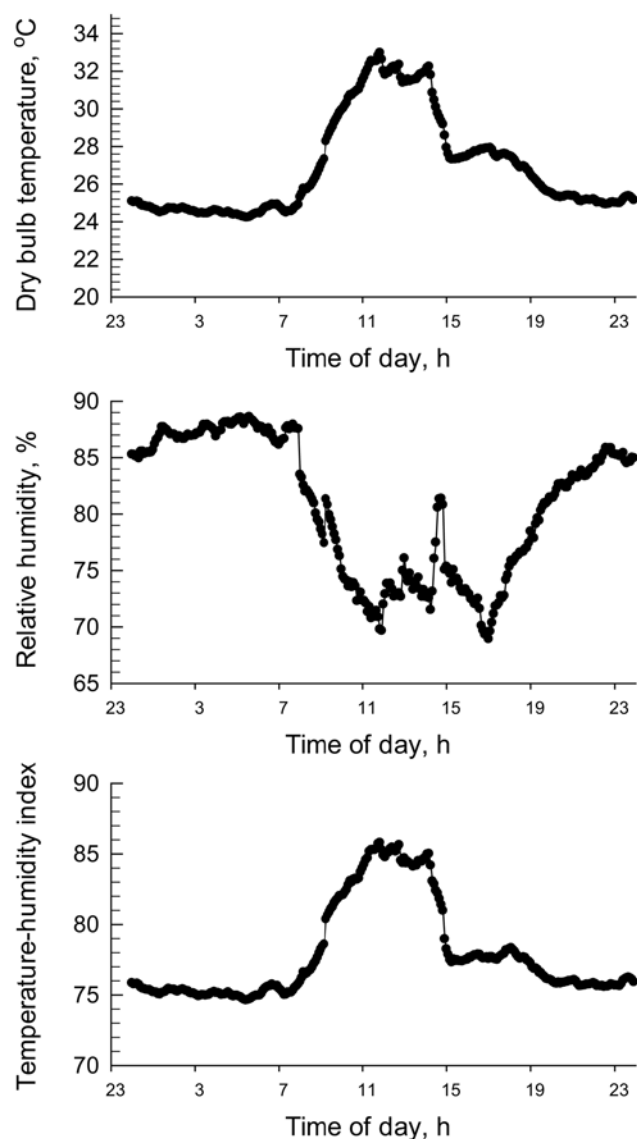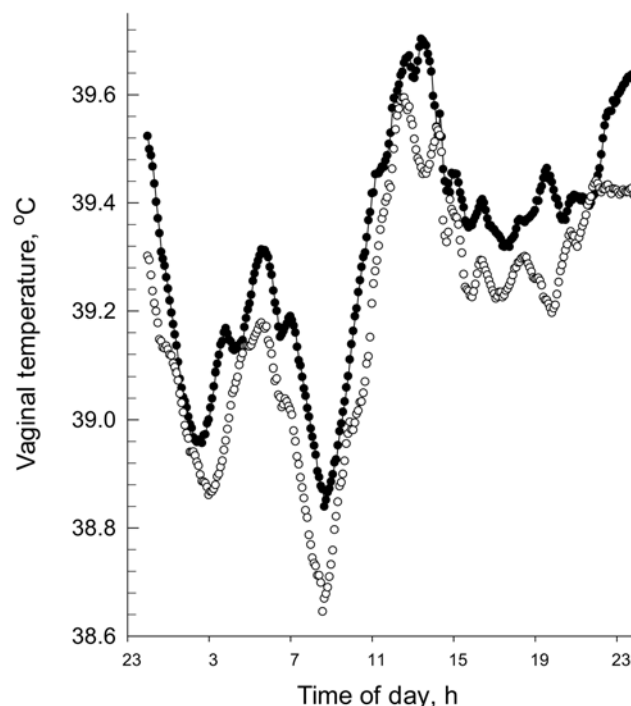

Figure 3. Daily variation in vaginal temperatures of wild-type (closed circles) and Larson Blue (open circles) as measured by iButton dataloggers (Maxim Integrated, San Jose, CA) every 5 min. Data are least squares means. The pooled SEM was 0.10°C for wild-type cows and 0.13°C for Larson Blue cows. Vaginal temperature was affected by time of day ($P < 0.0001$) but not by coat type ($P = 0.301$) or coat type × time ($P = 0.994$)
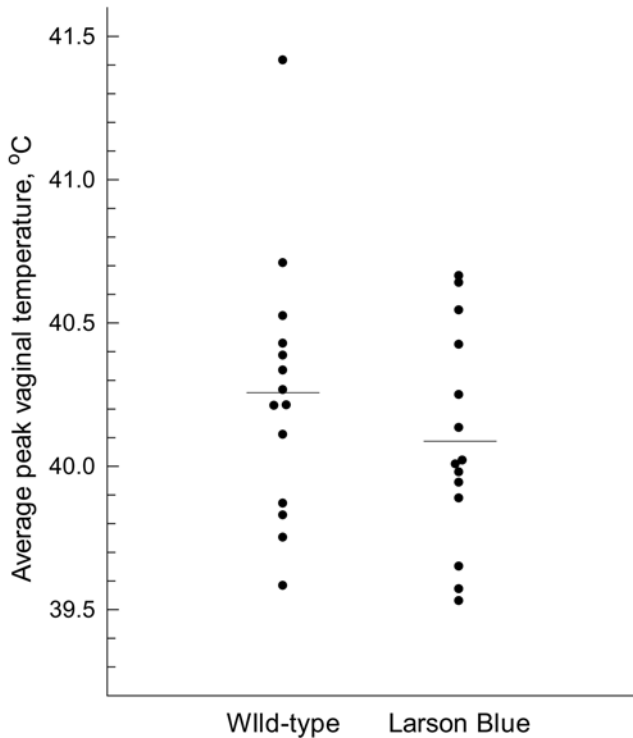
Figure 4. Average peak daily vaginal temperatures. Values for individual cows are represented by points. The least squares mean for each group is shown by the horizontal line. The effect of coat type was not significant ($P = 0.292$).

Figure 5. Milk yield per lactation in wild-type (closed circles) and Larson Blue (open circles) cows as affected by month of freshening. Data are least squares means ± SEM. Milk yield was affected by month of freshening ($P = 0.048$) but not by coat type ($P = 0.811$) or interaction of coat type × month ($P = 0.263$).

red/black coloration, none of the animals were positive for red color at one locus whereas 9 of 10 animals were not positive for red color at the other locus. The remaining animal was a heterozygote.

Whole Genome Sequencing

Two Larson Blue cows were analyzed with a sequence coverage of 8.5x and 9.9x. No variants with notable effects were identified in the coat-color genes listed in Table 1. Three variants with moderate effects on the *MLPH* gene are described in Table 2. One sequenced animal was heterozygous for all variants, and the second animal was heterozygous for 2 and homozygous for 1. The SnpEff annotation suggests that all 3 variants are associated with moderate effects that result in AA changes in the *MLPH* protein. These results are consistent with the hypothesis of dominant inheritance of Larson Blue coat color. However, tools for prediction of the simultaneous effect of 3 missense mutations on the MLPH protein are not available, so additional research is needed to confirm the effect of these AA changes on MLPH function. It is also not known whether the variants identified in *MLPH* exist in wild-type cows in the Larson herd. Furthermore, information about the inheritance of the phenotype is incomplete and it is not known whether the Larson Blue coloration exhibits dominance.
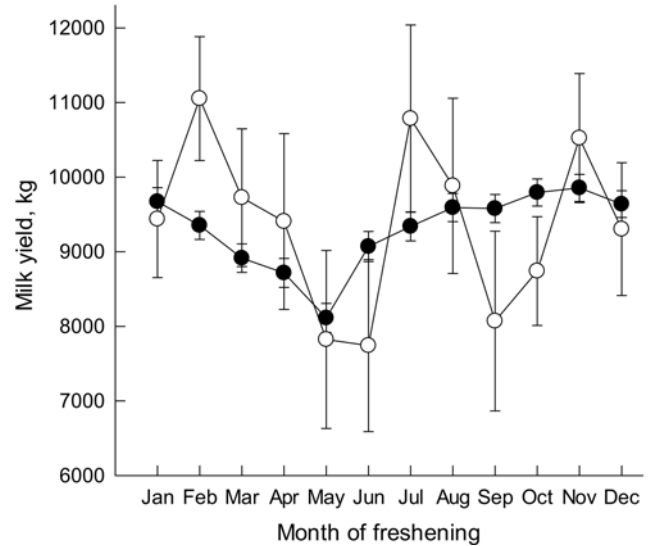
Li et al. (2016) recently described a frameshift mutation in *MLPH* that is associated with a novel coat color in Belgian Blue cattle. Given that changes in *MLPH* gene can result in coat color changes, it is possible that the observed changes in the cows sequenced may explain the color phenotype in Larson blue cattle. In any case, it is likely that a dilution gene is responsible for the phenotype. Several such genes have been described in cattle including *TYRP1* in the Dexter breed (Berryere et al., 2003) and *PMEL* in Highland and Galloway cattle (Schmutz and Dreger, 2013). However, these genes are not responsible for the Larson Blue phenotype (Table 1).

Vaginal Temperatures during Heat Stress

Environmental conditions during the experiment are shown in Fig. 2 and daily variation in vaginal temperatures is presented in Fig. 3. Time of day affected vaginal temperature ($P < 0.001$). Temperatures were elevated in the early morning and then declined to a nadir at 0800 to 0835 h (coincident with morning milking), rose thereafter to a peak at 1230 to 1330 h, and then declined. Cows were hyperthermic throughout most of the day, with vaginal temperatures only approaching 38.6°C around the nadir of temperature at 0800 to 0835 h. There was no effect of coat type ($P = 0.301$) or coat type × time ($P = 0.994$) on vaginal temperature. Overall, the least squares means of vaginal temperature were 39.3 (SEM 0.08) and 39.2°C (SEM 0.09) for wild-type and Larson Blue cows, respectively.

As a second measure of body temperature regulation, the average daily peak vaginal temperature was analyzed

(Fig. 4). There was no effect of coat type on peak vaginal temperature (P = 0.292): least squares means were 40.3°C for wild-type Holsteins and 40.1°C for Larson Blue Holsteins (SEM 0.11). With the exception of 1 wild-type cow experiencing an average peak vaginal temperature of 41.4°C, the range of temperatures was very similar for cows of both coat types (Fig. 4).

Milk Yield

Milk yield was affected by month of freshening (P=0.048) but there was no main effect of coat type on lactation milk yield (P = 0.811). The least squares means were 9,374 kg (SEM 388) for Larson Blue and 9,302 kg (SEM 236) for wild-type cows. There was no interaction between coat type and month of freshening (Fig. 5; P = 0.263). Milk yield was affected by parity (P < 0.001) but not by the coat type × parity interaction (P = 0.705). The least squares means were 8,392 kg (SEM 304; first lactation) and 10,357 kg (SEM 533; later lactations) for Larson Blue and 8,445 kg (SEM 170; first lactation) and 10,161 (SEM 179; later lactation) for wild-type cows.

It was hypothesized that Larson Blue cattle would be more thermotolerant than wild-type Holsteins because pigmentation of the hair is an important determinant of transfer of heat via solar radiation (Stewart, 1953; Finch et al., 1984). The data did not support the hypothesis because daily variation in vaginal temperature during the summer was similar for Larson Blue and wild-type Holsteins and there was no coat type × month of freshening interaction for milk yield. More broadly, these data are inconsistent with the idea that coat color is an important determinant of animal resistance to heat stress in dairy cattle reared in confinement. Cows in the present experiment were housed in free-stall barns with extensive shading, and it is possible that the Larson Blue phenotype would confer some thermotolerance when cattle are exposed to direct solar radiation, as, for example, in a grazing situation. Exposure to direct sunlight caused a smaller reduction in milk yield for predominately white Holsteins than for predominately black Holstein (Hansen, 1990).

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