Scientific Papers. Series D. Animal Science. Vol. LXVIII, No. 1, 2025
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THERMAL RESPONSE TO COLD STRESS IN TWO DIVERGENT STRAINS OF HOLSTEIN DAIRY CALVES – PRELIMINARY RESULTS

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Abstract

Cattle carrying the SLICK mutation (c.1382del; rs517047387) were shown to exhibit increased resistance to heat stress, with limited research on thermal response of carriers under cold stress. The objective of this study was to evaluate the response of un-weaned calves to cold stress exposure in two divergently selected Holstein strains, namely descendants of SLICK carrier bulls (experimental group - EG, n = 9) compared to those of Romanian Black and White bulls (controls - CG, n = 11). Calves were monitored during a cold weather episode of 3 consecutive days, with lower critical temperatures (<5°C for >12h/day), for infrared thermography (IRT), growth rates and cortisol levels. Average daily gains were of 792±110 g in the EG and of 928±150 g in CG group (P>0.05). IRT data showed significant differences between groups during the first day of the cold weather event, for both orbital (26.48±0.444°C in EG vs. 28.74±0.472°C in CG calves, P≤0.01) and nasal (18.23±0.820°C in EG vs. 21.05±1.200°C G in controls, P≤0.10) regions. Results suggest that calves sired by SLICK carrier bulls are exhibiting lower growth rates and have lower IRT-body temperatures in response to cold stress events.

***Key words**: cold stress, dairy calves, growth rates, infrared thermography, SLICK gene.*

INTRODUCTION

Europe is the fastest warming continent in the world, and as a result climate risks are threatening food security, ecosystems, water resources, farmers' financial stability and animals' productivity (EEA, 2024). The mean annual temperature in Europe was 2.1°C warmer in the last 10 years, compared to the pre-industrial period. As a result, climate change adaptation measures are critical to increase resilience and reduce risks in agriculture, including the dairy cattle sector.

Heat stress was shown to negatively affect cattle production, reproduction, health and general performance, leading to substantial economic losses (West, 2003; Wang et al., 2018). Moreover, heat tolerance in dairy cattle was found to have a negative genetic correlation with milk production parameters (Aguilar et al., 2009; Pryce et al., 2022).

In cattle, the prolactin receptor gene (PRLR), has undergone several mutations, collectively referred to as SLICK mutations. Cattle carrying at least one allele of the *SLICK* mutation have phenotypes characterized by a short, sleek hair coat (Dikmen et al., 2014).

SLICK mutations are prevalent in cattle breeds originating from the Caribbean basin and South America (Porto-Neto et al., 2018; Florez Murillo et al., 2021). The first SLICK mutation described was SLICK1 (c.1382del; rs517047387), found in the Senepol breed originating on the Caribbean Island of St. Croix (Littlejohn et al., 2014), which was recently introduced throughout introgression in the Holstein breed (Sosa et al., 2022).

Dairy cattle that are heterozygous for the SLICK1 mutation have demonstrated superior capabilities to regulate body temperature during intense heat stress exposure episodes (Eisemann et al., 2020; Landaeta-Hernandez et al., 2021).

The main significance of the SLICK1 mutation for the dairy industry is that carrier cows produce significantly more milk during intense heat stress, compared to cows without the mutation (Dikmen et al., 2014). While the

SLICK1 mutation was shown to improve thermotolerance to heat stress in carriers, consequences on the physiological responses under cold stress exposure are not yet documented.
The objective of this study was to evaluate the response of un-weaned calves to cold stress exposure in two divergently selected Holstein strains, namely descendants of SLICK1 carrier bulls, compared to those of Romanian Black and White bulls.

MATERIALS AND METHODS

The study was carried out at the Experimental Farm of the Research and Development Institute for Bovine Balotesti - Romania, between December 2024 and January 2025. Where two experimental groups were set-up, as follows: Group I - calves descendants of two SLICK carrier bulls crossed with Romanian Black and White cows (experimental group - EG, n = 9); Group II - purebred Romanian Black and White calves (controls - CG, n = 11). Both calves groups were managed under identical rearing conditions, and were monitored during a cold weather episode of 3 consecutive days, with temperatures of < 5°C, for +12 h/day (limits were corrected for air speed, and ranged between -3.9°C and +1.2°C), relative humidity 73-82% and wind speeds between 0.2 and 4.9 m/s. Temperature, humidity and air speed were recorded continuously using a Wi-Fi Sencor® (1xCR2032 model) weather-station, located in close proximity to the calves hutches. As reference values for setting-up the lower critical temperature values (LCT) for un-weaned calves, thresholds designed by AHDB UK (2024) were used.
Calves from the two experimental groups were balanced for age and body weight at the start of the experiment (EG 69.78±5.14 kg, CG 70.90±6.60 kg, $P = 0.8378$).
At birth all calves received 4 kg of colostrum per day, during their first 3 days of life and were kept in a maternity barn compartment. At 4-5 days of life, all calves were moved and housed in outside individual hutches (2.0 x 1.2 x 1.4 m), using deep straw bedding (1.5-2 kg/calf).
Suckling calves received a diet consisting out of 6 kg of milk, offered into two equal meals/day (at 12 hours intervals, 6:00 & 18:00). After the age of 10 days, calves had *ad libidum* access to fresh water, starter concentrates (18% CP) and alfalfa hay, until the age of 90±7 days, when weaning took place.
Infrared thermography (IRT) readings were taken using a FLIR ONE Pro LT mobile camera (19,200-pixel, range −20° to +400°C) and FLIR Systems INC© image processing software. Temperature measuring points were the lacrimal caruncle of the eye in the orbital region (*regio orbitalis*) and at the nasal region (regio nasalis), the IRT pictures being taken from a distance of 0.8–1.2 m in front of the calves (Figure 1), the three measuring times were at 6:00 AM (method described in detail by Mincu et al., 2023), right before the morning milk feeding.
Calves were weighted using a MP 800 - EziWeigh 7i® scale, at the beginning of the cold episode, at 7 and 28 days.
Blood samples were collected on the 3rd day of the cold exposure episode, and cortisol levels were analysed using the immune-enzymatic assay ELISA method (Stat Fax 2200-2600®).

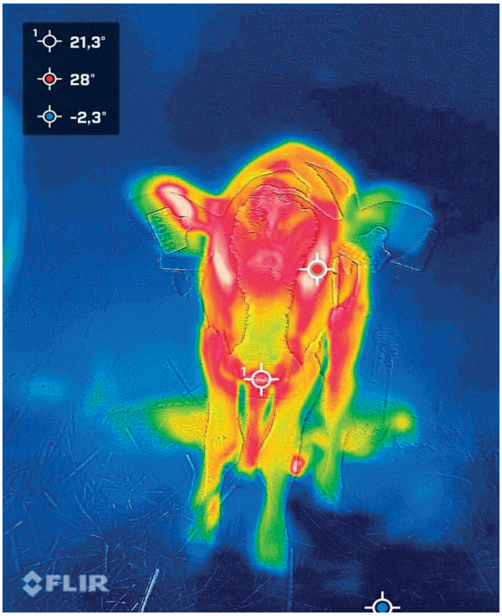

Figure 1. IRT reading for *regio orbitalis* and *regio nasalis* in a dairy calf during experiment (original photo)

Statistical analysis included descriptive statistics and the Mann-Whitney U test to determine differences between the two groups, using MiniTab17® software. Differences were considered significant at a level of $P \leq 0.05$, while the tendency for significance was set at $P \leq 0.10$.
The current experimental design was in accordance with the EU Directive 2010/63/EU on Experimental Animal Protection.

RESULTS AND DISCUSSIONS

Average daily gains of calves during the experiment were of 792±110 g/day in the EG and of 928±150 g/day in CG group (Table 1). Although the Romanian Black and White purebred calves distinctively outperformed the SLICK1 sired calves, differences were not supported statistically ($P>0.05$). The lack of statistical significance, could be attributed to the relative low number of subjects included in the study. However, differences in average daily gains of over 130 g/day are of importance, showing an impaired growth in SLICK1 sired group, given that the two groups had similar body live weights at the start of the experiment, and that they all received the same milk feeding diet.
Results on growth rates are in agreement with those previously published by Coffey et al. (2006), on differences in growth rates between divergently selected Holstein strains.
Hearth girth between the two groups was not significantly different ($P>0.05$) at the end of the experimental trial.
IRT data showed significant differences between the two groups during the 1st day of the cold weather event, for both orbital (26.4±0.44°C in EG *vs.* 28.7±0.47°C in CG calves, $P \leq 0.01$) and nasal (18.2±0.82°C in EG *vs.* 21.0±1.20°C G in controls, $P \leq 0.10$) regions (Table 2).
Furthermore, a tendency towards significance ($P = 0.0873$) was observed on 2nd day of the cold exposure event, for the nasal region. Generally, SLICK1 sired calves, had lower IRT readings for all three days of the experiment, in both anatomical regions.
Differences observed between the two groups during the 1st day of the naturally occurring cold event could be attributed to a delay in the activation of thermoregulation mechanisms in SLICK1 sired calves, in order to compensate for hypothermia.
Moreover, considering the innate ability of young ruminants to prevent a drop in the core body temperature, especially when severe hypothermia persists for extended periods of time, overcompensation triggering mechanisms could have been activated, leading to vasoconstriction as a reaction to prevents heat losses, and thus the SLICK1 sired calves had lower IRT readings, compared to the Romanian Black and White purebreds. This second hypothesis is supported by previous authors describing extensively the neonatal calves coping mechanisms to cold weather (Bertoni et al., 2020; Mota-Rojas et al., 2021).
Furthermore, our results regarding differences between the two anatomical measuring regions are consistent with those previously reported by Mota-Rojas et al. (2022), which found that IRT responses measured in various thermal windows differ, while the IRT temperature of the lacrimal gland shown the least temperature variation in bovines.
Blood cortisol levels were higher in SLICK1 sired calves, on average of 98.3±18.7 ng/ml, compared to Romanian Black and White purebred calves, which registered on average 67.6±14.9 ng/ml on the 3rd day of the cold weather event (Table 3). Although the blood cortisol levels in SLICK1 sired calves was on average higher 1.5 folded, compared to the local Holstein strain calves, there was no significant difference ($P>0.05$) between the two genotypes.
While cortisol levels in cattle are most commonly used as an indicator for welfare (Tamminen et al., 2021), being regarded as one of the main stress biomarkers, recent research by Kim et al. (2023) showed an elevation of cortisol levels in both beef calves and fattening steers, during cold stress exposure.
We are aware that the study has two limitations. The first is the less than desired number of subjects/calves included in the experimental groups, given that the SLICK1 mutation was just recently introduced in Romania. The second is represented by the short duration of the cold challenge, which might not elicit the full range of physiological responses in calves. To overcome these

shortcomings, for our future research trials on this subject, we plan to include a higher number of calves, as well as to monitor their health and growth performances for longer periods of time.

Our research trial suggests that when divergently selected cattle strains are introduced to new farming environments, significant attention should be given to un-weaned calves, in order to promote growth rates, general adaptation, health and animal welfare, taking into account practices such as the use of calf-jackets, increasing the daily milk intake, proper bedding and housing design.

Table 1. Averages and dispersion indices for average daily gain (g/day) and hearth girth (cm) in SLICK and non-SLICK un-weaned dairy calves during the cold stress period

Item	Genotype	Mean ± SEM	St. Dev.	Coef. Var.	Min.	Max.
Average daily gain (ADG, g/day)	SLICK	792±110	331	41.78	375	1250
	Non-slick	928±150	475	51.20	125	1750
Heart girth (cm)	SLICK	101.89±2.390	7.18	7.05	94.00	113.00
	Non-slick	102.20±3.420	10.81	10.58	85.00	94.00
Statistical differences						
ADG in SLICK vs. non-slick calves			P = 0.4840, *NS*			
Heart girth in SLICK vs. non-slick calves			P = 0.8697, *NS*			

Note: P>0.05 NS

Table 2. Averages and dispersion indices for orbital and nasal IRT data readings during the 3 days of cold weather exposure (°C)

Day	IRT Region	Genotype	Mean ± SEM	St. Dev.	Coef. Var.	Min.	Max.
I	Orbital	SLICK	26.48±0.444	1.332	5.03	24.70	28.40
		Non-slick	28.74±0.472	1.564	5.44	25.70	25.70
	Nasal	SLICK	18.23±0.820	2.460	13.49	14.10	21.40
		Non-slick	21.05±1.200	3.960	18.83	13.40	26.50
II	Orbital	SLICK	27.90±0.876	2.627	9.42	25.10	34.50
		Non-slick	29.245±0.920	3.051	10.43	23.20	33.30
	Nasal	SLICK	17.98±1.620	4.870	27.10	7.30	24.90
		Non-slick	21.51±1.240	4.100	19.05	12.70	26.90
III	Orbital	SLICK	33.28±0.858	2.573	7.73	28.90	36.10
		Non-slick	31.55±1.210	4.000	12.67	21.90	36.10
	Nasal	SLICK	24.02±1.970	5.900	24.55	16.50	33.50
		Non-slick	26.81±0.892	2.959	11.04	21.20	30.70
Statistical differences							
Day I, orbital region SLICK vs. Non-slick				**P = 0.0049, ****			
Day I, nasal region SLICK vs. Non-slick				P = 0.0681, *T*			
Day II, orbital region SLICK vs. Non-slick				P = 0.1596, *NS*			
Day II, nasal region SLICK vs. Non-slick				P = 0.0873, *T*			
Day III, orbital region SLICK vs. Non-slick				P = 0.3612, *NS*			
Day III, nasal region SLICK vs. Non-slick				P = 0.2871, *NS*			

*Note: P>0.05 NS; P≤0.01 **; Tendency towards significance (T) P≤0.10*

Table 3. Averages and dispersion indices for blood cortisol levels during the 3rd days of cold weather exposure (ng/ml)

Item	Genotype	Mean ± SEM	St. Dev.	Coef. Var.	Min.	Max.
Blood cortisol level (ng/ml)	SLICK calves	98.3±18.7	56.2	57.15	30.7	182.4
	Non-slick calves	67.6±14.9	47.2	69.78	26.1	144.5
Statistical differences						
Differences in blood cortisol levels in SLICK vs. Non-slick calves				0.3689, NS		

Note: P>0.05 NS

CONCLUSIONS

The evidence from this study suggests that calves sired by SLICK1 carrier bulls are exhibiting lower growth rates, have lower IRT-body temperatures, and show more elevated levels of blood cortisol in response to cold stress events, compared to purebred calves from the local Romanian Black and White Holstein strain.

This study is the first step towards enhancing our understanding on SLICK1 sired calves adaptation to temperate climatic conditions, with a focus on physiological responses to cold stress events during the first 3 months of age.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Romanian Ministry of Agriculture and Rural Development, project number ADER 7.1.9/2023.

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