BRIEF COMMUNICATION: Influence of the 'SLICK' allele of the prolactin receptor gene (PRLR) on the growth and behaviour of dairy heifers during winter.

LA Robb [1]*, RH Bryant[1], BP Beckett[1], EG Donkersloot[2], GM Worth[2], Huitong Zhou[1], and JGH. Hickford[1]
[1]*Faculty of Agriculture and Life Sciences, Lincoln University, PO Box 85084, Lincoln 7647, Christchurch, New Zealand.*
[2]*Livestock Improvement Corporation Ltd., Newstead, Hamilton 3240, New Zealand*
**Corresponding author.* Email: Luke.Robb@lincolnuni.ac.nz

Abstract
This study investigated the effects of the SLICK allele on the responses of dairy heifers in cold conditions in New Zealand. Three groups (n=9/group), control, non-SLICK and SLICK progeny of bulls heterozygous for the SLICK allele were investigated. These heifers were grazed together to identify their responses to winter conditions under adequate, followed by restricted feed allocation. The control group had an 87% lower growth rate during the restricted period compared to the SLICK group (0.064 vs 0.484 kg/d, P = 0.035). During restricted feeding conditions, the SLICK heifers showed improved pasture harvesting efficiency, by maintaining growth rate while having a lower eating time, compared to the control heifers (277 vs 317 ± 10.9 mins/day respectively, P = 0.017). The SLICK group were also less active than the control group. These differences indicate more research is needed to understand the wider role of the prolactin axis on the physiological responses of SLICK cattle.

Keywords: Heat stress; Cold challenge; Dairy cattle; SLICK allele; Prolactin receptor gene.

Introduction

Despite a temperate climate, New Zealand will be affected by climate change and an increased potential for extreme seasonal weather events, with the number of tropical nights (defined as when the daily temperature does not fall below 20°C) to increase an average >1000% nationally and >2000% in regions like Canterbury and Taranaki from 2024 onwards (Abercrombie et al. 2018). Breeding for improved heat tolerance in livestock is one approach to ensure future welfare and productive performance under warming conditions. In 2014, a genetic variant known as the SLICK allele was introduced into Holstein-Friesian Jersey crossbred cattle to ascertain its relevance in a temperate climate dairy industry. The SLICK allele is caused by a single base pair deletion in the prolactin receptor gene (*PRLR*), originating in Senepol cattle (Littlejohn et al. 2014). When investigated under New Zealand heat load conditions, SLICK carrier Holstein-Friesian cattle displayed improved thermoregulation (Worth et al. 2023).

The short, sleek coat of SLICK cattle has been described as an important contributor to their heat tolerance (Dikmen et al. 2014), but little is known about whether SLICK carrier cattle can or need to adapt their behaviour and/or physiology, when facing a cold challenge. The phenotypic advantage in periods of heat challenge may therefore potentially be disadvantageous in the winter. This would be of concern for New Zealand's typically outdoor cattle systems, where only 85-89% of heifer replacements reach target live weight by their first calving (McNaughton and Lopdell, 2013). Accordingly, the purpose of this research was to investigate if the presence of the SLICK allele influenced heifer growth rates, behaviour, or other dairy cattle traits in winter conditions.

Materials and Methods

Experimental site and design

The study was conducted with approval from the Lincoln University Animal Ethics Committee (#2023-17) at the Lincoln University Research Dairy Farm (-43.64, 172.46) between May and July 2023. The experiment was a randomised design with three treatment groups, and nine heifers per treatment. The control heifers were the progeny of AI sires selected for high breeding worth, while the non-SLICK group consisted of heifers born to AI sires from LIC that were heterozygous for the SLICK allele, but did not inherit the SLICK allele, and the SLICK heifers were the progeny of the same carrier bulls carrying the SLICK allele. All heifers were approximately 10 months of age and had an average breeding worth (daughter proven) of \$234 ± 27.4, \$125 ± 67.7 and \$125 ± 95.5 (mean ± sd) for the control, non-SLICK and SLICK respectively.

The absence or presence of the SLICK allele in the heifers was confirmed using DNA extracted from ear blood samples (Zhou et al., 2006) using a polymerase chain reaction-single strand conformation polymorphism approach.

All the heifers had been under the same management from birth and had been reared together on pasture since weaning. On 13 June 2023, the treatment heifers were drafted from a larger group of heifers and allocated 28 m^2/animal/day of perennial ryegrass and white clover pasture using temporary fencing. During the experimental period, supplement was fed as pasture baleage at 1 kg DM/head/day. Between 13 and 27 June was the unrestricted feed period where all heifers were offered approximately 3.5% of bodyweight as pasture (above a 2cm residual). This was reduced to an allocation of 2.8% of bodyweight between 27 June and 11 July as the restricted feeding period. The restricted feed period was used to reduce the energy available for metabolic heat production to increase the physiological factors which lead to cold stress, in response to the milder climatic conditions than anticipated. The allocation area and supplement remained the same during the entire observation, but during the restricted period the pre-graze pasture mass reduced from 2550 ± 194.8 to 2080 ± 69.9 kg DM/ha (± SEM).

Measurements

Live weight was measured (un-fasted) on four occasions: a covariate weight taken on 15 May, and again when the heifers were drafted on 13 June, 27 June, and 11 July 2023. Behaviour was monitored between 22 June to 5 July, by attaching an ear mounted accelerometer (Senztag-NZ, Morrinsville, NZ) to six SLICK and five control heifers. The tri-axial accelerometer tags recorded minutes per hour for movement that is categorised as 'high activity', 'active', 'non-active', 'eating', and 'rumination' (Pereira et al. 2017).

Apparent dry matter intake was estimated using the pasture disappearance method using a calibrated rising plate meter (RPM, Jenquip, Feilding, New Zealand). Baleage dry matter content was determined from weekly subsamples of fed baleage, and refusals determined by visual estimation.

Climate data were collected from the NIWA CliFlo database, Broadfields weather station (approximately 2km from the experimental site; ID 17603). A temperature humidity index (THI) was calculated using the equation described by Davis et al. (2003):

$$THI = 0.8T + [RH * (T - 14.4)] + 46.4$$

where T is daily average temperature and RH is relative humidity.

Statistical analysis

The statistical analyses were conducted using GenStat (V. 22.1.0 VSN International Ltd). Growth rate, live weight, and behaviour (minutes/day per category) were compared using the general linear model procedure in GenStat. The heifer group and period (unrestricted and restricted) were included as fixed terms and animal as the random term. Pre-winter live weight on 15 May was included as a covariate. Behaviour data (minutes/hour) for the five categories of behaviour were analysed using repeated measures procedure in GenStat, with group and hour of the day (and their interaction) as fixed terms, and individual heifer as the random term.

The daily average temperature between 22 June and 11 July was 8.8 ± 0.89 °C and ranged between 4.1 and 13.4°C. The daily average temperature was 2.5 °C ($P = 0.011$) cooler than the 30-year average of 11.3 ± 0.25 °C (1992-2022). The average THI-values during the unrestricted and restricted periods were 49.8 ± 1.30 and 42.2 ± 1.70 °C respectively. According to Fu et al. (2022) cold stress is more likely to occur at THI < 38, and during the observation THI below 38 occurred on 2 out of a total of 14 days indicating limited cold challenge.

Pasture and supplement utilisation was 85 and 95% respectively resulting in a total daily apparent intake of 7.4 ± 0.48 and 5.8 ± 0.31 kg DM/heifer/day, in respective unrestricted and restricted periods. Restricting feed intake increases sensitivity to cold conditions by restricting the energy available for metabolic heat production (Wang et al. 2023). While we could not confirm if the heifers in the current experiment were experiencing cold stress, it is evident that they altered their behaviour (inactive and rumination time) in response to the change in nutritional and climatic conditions. Collectively the heifers (control and SLICK) increased rumination time from 230 to 290 ± 17.7 minutes/d between the unrestricted and restricted period ($P<0.05$). Eating time stayed the same between periods, but control heifers spent more time eating compared to SLICK (317 vs 277 ± 10.9 mins/d, $P < 0.05$). There was no difference between groups or periods for active (sum of active and high active) behaviour which was an average 437 mins/d. However, heifers spent more time inactive in the unrestricted compared with restricted period (460 vs 435 ± 7.4 mins/d respectively, $P < 0.05$) and SLICK heifers were more inactive than control heifers in both periods (468 vs 427 ± 7.4 mins/d respectively, $P = 0.001$, Table 1). Inactivity, in the form of recumbency, can be used as a heat and energy conservation method for young stock, primarily to reduce the surface area exposed to air. This reduces the amount of maintenance energy needed to maintain temperature homeostasis in cold conditions (Rawson et al. 1989).

Results and discussion

Table 1. Daily behaviour (minutes/day) of heifers comprised of CONTROL (n=6), and SLICK carrier (n=5) groups. Where SEM is the standard error of the mean.

	Non-restricted		**Restricted**					
	CONTROL	**SLICK**	**CONTROL**	**SLICK**	**SEM**	**Group**	**Period**	**G x P**
Eating	319	266	315	287	10.9	**0.017**	0.527	0.423
Ruminating	223	237	275	305	17.7	0.401	**0.025**	0.744
Active	203	208	188	158	14.6	0.536	0.113	0.405
High active	257	253	241	239	8.4	0.795	0.226	0.959
Not active	438	482	416	454	7.4	**0.001**	**0.029**	0.777

By the end of the study period live weight increased from 230 to 244 ± 1.0 kg between the unrestricted and restricted periods ($P < 0.001$), although there was no interaction with group for live weight ($P = 0.137$). The impact of cold and feed restriction on growth was a pronounced reduction in growth rate from 622 to

299 ± 56.4 g/d between the unrestricted and restricted period. There was a group-by-period interaction for growth rate ($P < 0.001$) in that the control heifers experienced much greater weight gain reductions than either of the other groups (Figure 1).

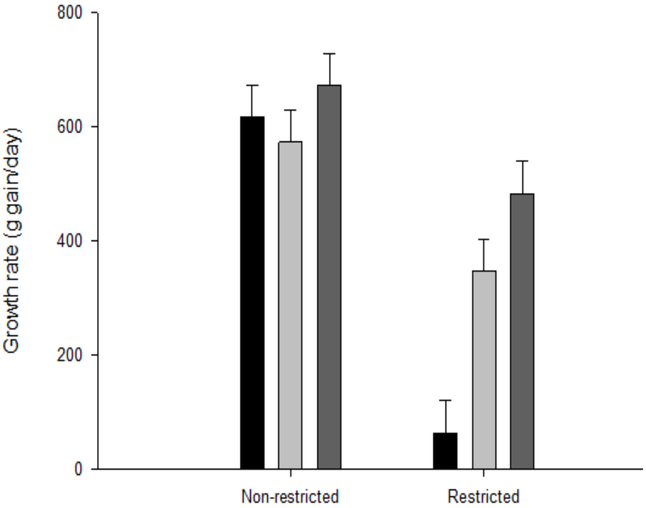

Figure 1. Growth rate (g/d) of heifers from different sires including control (black) versus non-SLICK (light grey) or SLICK (dark grey) and different feeding restriction during winter. Bars represent the standard error of the mean.

The dry matter intake requirements of cattle have been reported to be increased in periods of colder weather, compared to warmer periods (Wang et al. 2023), and based on the change in growth rate between the two periods, the control heifer's maintenance requirements increased (demonstrated by the 90% decrease in growth rate). In contrast, the SLICK heifers maintained (statistically) the same growth rate as in the unrestricted period, indicating that they were better at regulating heat loss as demonstrated by the higher inactive time.

Green et al. (2013) reported that the most efficient heifers spent less time eating per day compared to the least efficient heifers ($P = 0.026$). This would indicate that the SLICK heifers are able to be more efficient with the feed they eat and achieve this by modifying their grazing behaviour to spend more time eating during the restricted period. They were able to outcompete the control heifers for enough energy to maintain their growth rate.

We accept the small sample size and short duration of this study which limits our conclusions. However, as a pilot study these results indicate that SLICK heifers modified their behaviour to adapt to the lower feed allocation and conserve energy during cold conditions, which enabled them to outcompete control heifers. These results highlight the need for further investigation into behavioural adaptations to environment associated with breeding. The concept of behavioural genetics has previously been reported among beef animals (Garcia et al 2024) and our results indicate that SLICK heifers -in contrast to control - were able to maintain growth rates during a period of mild cold challenge and feed restriction. Further investigation into the behavioural differences of SLICK dairy animals, under a range of environmental conditions is warranted.

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