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Measuring emotions in dairy cows: Relationships between infrared temperature of key body parts, lateralised behaviour and milk production

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ABSTRACT

The emotions of cows could be reflected in their body temperature, productivity and behaviours. We tested whether there is a positive correlation between non-invasive infrared temperature (IRT) and right-side laterality (presumed to indicate anxiety), and negative correlation with milk production in a large group of randomly selected cows. Lactating cows (n = 171) were examined individually in 3 consecutive one-month periods. Each month maximum infrared temperatures of the head and coronary bands of forelimbs were recorded daily for 6 days and lateralisation was determined from ten daily forced lateralisation tests. In the first month right-lateralised cows had higher IRT in their eyes than left-lateralised cows. The absence of an association between IRT and laterality in periods 2 and 3 suggests habituation to the test conditions. Right lateralised cows waited for longer before milking than left lateralised cows, and limb IRTs were negatively related to the waiting time before milking. We conclude that the connection between lateralised behaviour and eye IRT in this study provides evidence of differences in cows' emotional response to the forced lateralisation test.

1. Introduction

In a commercial dairy herd, cows may display negative emotions, such as fear, apprehension, stress and anxiety. This may be due to rough handling by novel persons, unsuitable social grouping or isolation, transportation, climatic extremes, feed alterations, and the close of proximity of dominant herd mates (Phillips et al., 2015; Goma et al., 2018). Fear motivates animals to react to the perceived threat or changes in the environment, with stress being the origin of the psychological, physiological and behavioural responses of animals that facilitates the reaction to the threat to enable it to occur as early as possible (Mills et al., 2010). Chronic hyperarousal brought on by anxiety results from a persistent state of dreading an unpredictable contextual threat (Grillon, 2002; Barlow et al., 2004; Goma et al., 2018; Lee et al., 2018). Negative emotional reactions in cows often result in decreased appetite (Osei-Amponsah et al., 2020). Reduced appetite in cows lowers their reproductive and/or productive performance (West, 2003; Bernabucci et al., 2010), while increasing abnormal behaviour (Lee et al., 2018), and weakening the immune system (Fell et al., 1999), which increases their risk of developing mastitis and other infectious and/or metabolic diseases (Goff et al., 2002). To maintain good health, welfare, and production, it is crucial to identify cows who are experiencing negative emotions. This will facilitate targeted interventions.

The most commonly used measure of negative emotional responses is cortisol concentration, usually in blood plasma (Beerda et al., 2004), but also, in milk (Verkerk et al., 1998), saliva (Morrow et al., 2002), urine (Pol et al., 2002), and faeces (Morrow et al., 2002). In addition, anxiety responses have been measured by behaviour tests such as flight speed, crush score, the forced lateralisation test, and the human approach test (Kilgour et al., 2006; Gibbons et al., 2011; Phillips et al., 2015; Hulbert and Moisá, 2016; Lee et al., 2018). Motor laterality refers to asymmetric motor patterns in the body. In a forced lateralisation test for cattle, the side of passing a human in a forced lateralisation test is correlated with behavioural indicators of their emotions. Specifically, cows passing on the right side of the person are more likely to defecate whilst passing, to pass singly, rather than in pairs, and are less likely to look at the person

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when passing (Goma et al., 2018). These tests do have drawbacks, though, as they are labour-intensive, lab-reliant, and necessitate the active participation of animals, skilled workers, and specialised facilities. Additionally, behaviour responses to emotions vary considerably within and between animals (Clark et al., 1997), hence frequent and rapid measurement is more likely to reflect emotional states. Non-invasive and/or remote devices have been used to measure heart and respiratory rates, foraging behaviour, lying, standing, and walking behaviour, and heart rate (Lefcourt et al., 1999; Eigenberg et al., 2000) in an effort to reduce these limitations. These non-invasive and/or remote devices also have drawbacks such as but not limited to the high cost to purchase and fitting the devices to the animals, ensuring proper restraint and handling, as well as the potential negative effect of the devices themselves on cow behaviour and emotions as an additional apparatus to carry on (Stewart et al., 2005).

An alternate strategy to estimate emotional responses is through the remote measurement of body temperature, as short-term or sustained emotional change leads to increases in blood flow within external parts of the body (Proctor and Carder, 2016; Tamioso et al., 2017; Unruh et al., 2017). By using an infrared sensor to measure the heat emitted from external body surfaces like the eyes and the coronary bands of limbs, infrared thermography recognises that changes in the heat emitted are proportionately correlated with changes in body temperature (Jenkins et al., 2009). In previous studies (Montanholi et al., 2008; Alsaaod and Büscher, 2012; Schaefer et al., 2012; Stokes et al., 2012; Church et al., 2014; Tamioso et al., 2017), an association between behaviour, IRT of external body surfaces, and feed efficiency in cattle has been found. Based on extreme left (non-anxious) and right-side (anxious) laterality (as suggested by Phillips et al., 2015; Goma et al., 2018), Uddin et al. (2019) found a positive correlation between the maximum IRT of the eyes and the coronary band of the forelimbs with right side (anxiety-related) laterality. In a similar study by Uddin et al. (2021), tests of random cows (38, 37 and 41 cows in period 1, 2 and 3, respectively) were conducted over 3 periods, along with flight speed, crush score, and rectal temperatures, in addition to a forced lateralisation test, and milk production and IRT measures. IRT measures were highly repeatable, had a positive association with anxious behaviour, such as sniffing, but not in a consistent pattern over the 3 periods. However, Uddin et al. (2021) did not find an association between IRT measures and laterality, which encouraged us to further investigate this in randomly selected lactating cows using a large sample size and in a repeated period of experiments. We hypothesized that an association between IRT, behaviours in a forced lateralisation test and milk productivity will be more evident in larger sample size of randomly selected lactating cows. Thus, this study evaluated the behaviours exhibited during forced lateralisation tests, IRT and milk productivity of 171 cows in 3 one month periods. The objective of this study was to determine the relationship between infrared temperature, lateralised behaviour and milk production of dairy cows over a prolonged period of their lactation.

2. Materials and methods

The Animal Ethics Committee of the University of Queensland in Australia granted approval for this study (SVS/365/17). Details of the experimental procedures has been described in Uddin et al. (2021). This study evaluated IRT measures (thermograms captured during milking in the parlour) and milk production records during the same 6 days, and then behaviour (video recorded in a forced lateralisation test while cows were leaving parlour to the feeding area after the afternoon milking) on 10 consecutive days, started one day after the 6 day thermogram capturing in each period of 171 lactating dairy cows in 3 periods. This included the cows evaluated in Uddin et al. (2021).

In brief, two groups of cows: 79 % were Holstein-Friesians and the remainder Jersey, Brown Swiss, Red Swiss and mixed breeds (early lactating: n = 135, and late lactating: n = 115), identified in the first day of experiment (Period 1), were reared and managed in a subtropical southern hemisphere environment. As they progressed through lactation (days in milk, DIM), they switched from the first to the second group and decreased their milk output to about 17 L per cow per day. They were milked twice a day in a covered, rapid exit, herringbone parlour (GEA, Westfalia Surge Australia Pty Ltd, 16 Trade Park Dr, Tullamarine VIC 3043), with 14 cows per side, in the morning from 5:00–8:00 h and in the afternoon from 15:00–18:00 h. A waiting yard with a sprinkler system was attached to the milking parlour where the cows gathered before milking.

When the outside temperature was 30 °C or higher, water was automatically sprayed on all the cows for 1 minute in every 5 minutes. This did not happen on any day in period 1, but in periods 2 and 3, the sprinkler was used every day except day 5. Cows were kept in a covered feedlot area after morning milking and given a mixed ration during the hottest parts of the day while being allowed to graze on subtropical pastures during the cooler parts of the day and night (from 14:00–07:00 h). On a dry matter basis, we calculated that the diet should consist of roughly 66 % mixed ration and 34 % pasture to provide the necessary nutrients for the herd's intended level of milk production. A mixer wagon delivered the mixed ration to the cows at two 60 m feed bunks (providing 52 cm of trough space per cow) and two 5 m water troughs (providing 4.3 cm of trough space per cow), which were separated by a central concrete passage. The mixed ration was based on maize silage with milled cereal grains, high protein meal, and a vitamin and mineral premix. The examination of cows in each period began chronologically with infrared thermography and daily recording of lactation variables between d 6 and 11. A forced lateralisation test (FLT) was performed after every afternoon milking between d 13 and d 22 (Table 1).

2.1. Infrared thermography

This procedure has been described in Uddin et al. (2021). Briefly, a single thermogram captured from each cow a measurement of its head, and its lower forelimbs infrared temperature at roughly the same time each day (15:00–18:00 h) during milking in the parlour. Thermograms of head and limbs of a cow were captured within a 30 s time frame in a given day. Each thermogram was taken directly in front of the cow at a distance of 1 m from the camera's lens (FLIR E40, FLIR Systems, Inc. Wilsonville, USA) and at a height of about head height (approximately 1.5 m). The camera was factory calibrated to 160 $\times$120 (19,200) pixels, 18 mm focal length and had an infrared resolution of 0.07 °C and thermal sensitivity across the range −20 °C to +650 °C. The thermogram of the lower forelimbs was then taken by lowering the camera. The same person in identical green overalls took all of the thermograms. Thermograms were automatically stored on a memory card (8 GB SD, T911650ACC, FLIR Systems, Inc. Wilsonville, USA) that was attached to the camera. These thermograms were then transferred to a desktop computer. Using FLIR Tools software, the maximum IRTs of the eyes and coronary band of the forelimbs was recorded, provided that maximum IRT of eyes and the coronary band of forelimbs has been identified as most representative of thermoregulatory changes than means and or minimum by Uddin et al. (2019) (Fig. 1; a and b, Table 2).

The camera was calibrated manually to emissivity 0.98, which is typically thought of as the emissivity of biological tissues (Steketee, 1973), and reflected temperature 20 °C (as advised by the manufacturer) before taking the thermograms. To ensure that all imaged body parts were in the shade, a meshed shade cloth awning was installed on the right side of the milking parlour beginning one week before the start of the infrared thermography and remaining in place throughout all sessions. Using a Kestrel 4000 Pocket weather station (made by Nielsen Kellerman Australia, ACT, Australia), ambient temperature and relative humidity of the milking parlour area were measured from three different locations (each end and the middle of the two sides of the building where the groups of 14 cows were milked). The data means were input into the FLIR Tools software's in parameters section, which modifies the

Table 1
Actual day of each test (IRT = thermal images capturing; FLT = recording of cow behaviour in forced lateralisation test) in a period/month, across 3 periods/ months.

Day in month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	..	..	30
IRT						√	√	√	√	√	√														
Milk data						√	√	√	√	√	√														
FLT													√	√	√	√	√	√	√	√	√	√			

thermogram's IRT for background temperature and relative humidity (FLIR Systems Inc, Wilsonville, USA).

2.2. Milk production and quality

This procedure has also been described in Uddin et al. (2021). Briefly, the rapid exit herringbone parlour (GEA, Westfalia Surge Australia Pty Ltd, 16 Trade Park Dr, Tullamarine VIC 3043) and associated herd management software (GEA, Westfalia Surge Australia Pty Ltd), recorded the milk yield of each cow at morning (a.m.) and afternoon (p.m.) milking to the nearest 0.2 kg, as well as the times of attachment and removal of milking cups. The DIM and parity of each cow has been recorded from the herd management software, separately for each day of experiment in each period/month. The daily milk yield was calculated as the sum of the a.m. and p.m. milk yields, averaged over 6 d periods.

The difference between the time the milking cups were attached and the time the cows entered the waiting yard for afternoon milking was used to calculate the waiting time for each cow. The milk yield at p.m. milking in kilograms was divided by the milking time (the interval between applying and removing the milking cups, in seconds), was used to determine each cow's milk secretion rate. According to monthly measurements taken one day after the 6 day thermogram capturing period, milk fat and protein content as well as somatic cell count (SCC) per cow were calculated. In order to collect individual cow composite milk samples from the morning and afternoon milking, milk sampling meters (n-Farm; GEA, Westfalia Surge Australia Pty Lt d, 16 Trade Park Dr, Tullamarine VIC 3043) were used. Milk samples were put into vials with a milk preservative and sent to an industrial milk analysis lab (Australian Herd Recording Services, Nundah, QLD 4012, Australia) so that the milk fat and protein content could be measured by mid-infrared spectroscopy and the SCC could be determined by flow cytometry.

2.3. Forced lateralisation test (FLT)

This procedure has been described in Uddin et al. (2021). Briefly, the FLT, or forced lateralisation test, was conducted to observe cow behaviours as they exited the milking parlour and made their way to the feeding area after the afternoon milking. The study took place over a period of 10 consecutive days, starting 1 day after thermograms were captured. During the study, videos were recorded of the cows' behaviours using a Sony Cyber-shot video camera held by a person who had previously collected IRT data in the parlour. The person stood in the middle of the 12.0 ×4.3 m lane and held the camera in both hands in a fixed position, looking straight ahead as the cows approached and passed by. To ensure that the cows were not familiar with the person recording the videos, the person changed appearance each period. During the first period, the person wore green overalls and a cap, during the second period, person wore sunglasses, and during the third period, the person wore a backpack and sunglasses. Different responses to a variety of coloured clothing have been reported in cattle (Rybarczyk et al., 2003). We assumed from this that the extra glasses and backpack would make the cows think it was a different person. This helped to eliminate any potential bias in the cows' behaviours towards a familiar or unfamiliar person.

The laneway was bordered by metal rail fencing and had no cues that encourage laterality, such as worn routes on one side. Recording of behaviour began when each cow approached within 6 m of a person marked by a white line sprayed on the lane and continued until the cow disappeared from camera view.

The study measured several behaviour variables, including laterality index, stopping while walking, sniffing, standing, walking speed, and tail position. These behaviours are identified as either present (1) or absent (0) during each day's video recording. The laterality index of each cow was calculated as the $\log_{10}$ transformed ratio of the sum of the cow's left and right side passing the person in the laneway, with 1 added to both the numerator and denominator to avoid 0 being a numerator or denominator.

A laterality index of $\leq$1.05 indicates a left lateralised cow, which is presumed to be less anxious, while an index of $\geq$−1.05 indicates a right lateralised cow, which is presumed to be more anxious, according to previous studies (Phillips et al., 2015; Goma et al., 2018). Overall, the study aims to investigate how the presence or absence of visual cues affects the behaviour of cows in a concrete laneway.

2.4. Statistical analysis

The maximum IRT of each measured external body surface region was averaged across days in the period for each cow prior to analysis. Behaviour variables of the FLT of each cow were summated over 10 days in a period. The preferred side of passing was transformed to $\log_{10}\{(L+1)/(R+1)\}$ in each 10-d period of the FLT measurement. Analysis of variance (ANOVA) for all variables (IRT, milk productive, and behaviour) was used to determine significant factors ($P < 0.05$), with means presented with standard errors of the difference between two means (SED). Residuals of ANOVA tests were tested for normal distribution by the Kolmogorov-Smirnov tests. Variables whose residuals were not normally distributed were analysed by a nonparametric test (Mood Median Test) and presented as median values and numbers of cows above, below and at this value.

In each period, the variables were analysed with a stepwise forward-backward linear regression procedure (tested at significance level $P < 0.10$) as in Uddin et al. (2019) to determine the best potential prediction equation, in Minitab (Version 18; Minitab Inc., State College, PA). In the stepwise regressions, the outcome variables were the IRT measurements (Y), and the predictors were the behaviour and production related variables, including age and parity of cows. An α of 0.10 was used to enter or remove the predictors for the stepwise regression. The stepwise regressions included the side of passing as a ratio, $\log_{10}\{(L+1)/(R+1)\}$. Residuals of the regression equations were normally distributed by the Anderson-Darling test ($P>0.05$), and the variance inflation factors of the variables were not more than 2.5.

3. Results

3.1. The effects of laterality on IRT, milk productivity and behaviour variables

3.1.1. Productivity variables

Right (R) side passing cows had higher IRT in both their left eye and right eyes (and therefore both eyes), compared to left (L) side passing cows, but only in period 1, not periods 2 or 3 (Table 3). There were no differences in IRT of the limbs, the ratio of eye to limbs or left to right eye between R and L side passing cows in any period (Table 3).

L cows tended to be older than R cows, which was significant in period 3 (Table 4). R cows had lower DIM than L cows in period 1. SCC

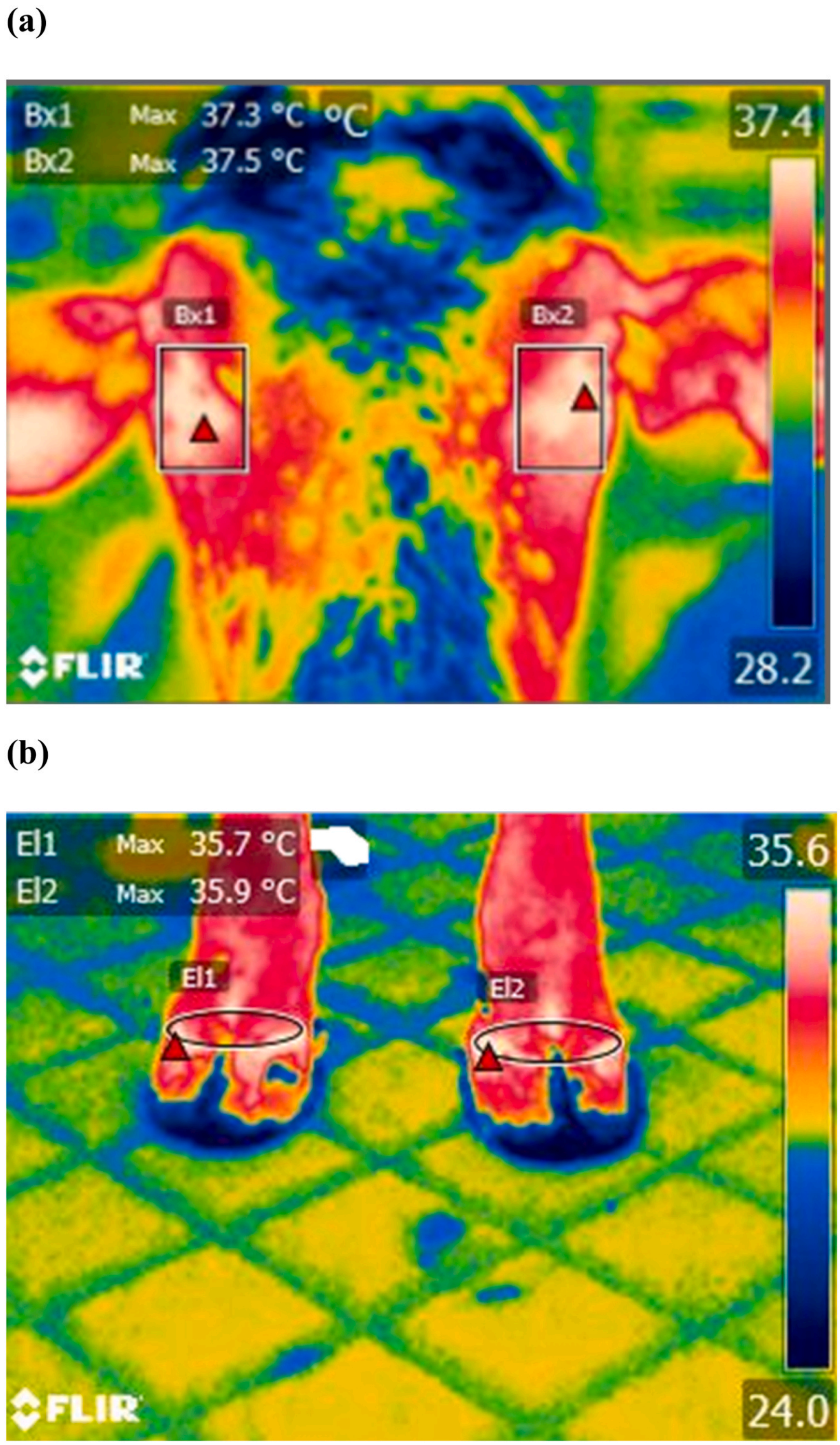

Fig. 1. Thermograms of a cow's head and lower forelimbs, seen from the front (a and b). Thermograms depict the external body surfaces of the right and left eyes, as indicated by the boxes in (a), as well as the coronary bands of the right and left forelimbs, as indicated by the ellipsoids (b), respectively. The pixel with the highest infrared temperature is marked by a red triangle within each shape. The infrared temperatures are represented by the coloured index scale on the right side of thermograms, going from the hottest (white) to the coldest (blue), through red, yellow, and green. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

was higher in L cows compared to R cows in periods 1 and 2. In contrast, the milk secretion rate was higher in the R than L cows in period 2 (Table 4).

3.1.2. Behaviour variables

In the third period, there were relatively fewer L cows above the median value for stopping while walking and a lower median value for raised tail (Table 4), compared with R cows. In the first period there were more L cows with a high median value for relaxed tail. In the first period there was more sniffing by L cows and relatively fewer L cows with a slow speed of walking. In periods 1 and 3, waiting time was higher in R cows than L cows.

Table 2
Definitions of infrared temperatures of the external surfaces of the body; and behaviours observed during the forced lateralisation test.

Item	Description
Infrared temperatures	
Left or Right eyes	The area around the eye, comprising the orbit and periorbital area, that stretches from the outer to inner canthus.
Both eyes	(Region from the outer to inner canthus of the left eye plus region from the outer to inner canthus of the right eye)/2}.
Left or Right limb	The heavily vascularised band of tissue that is thicker and extends just above the forelimb's specific coronary band.
Both limbs	(The thickened band of highly vascularised tissue that is located above the upper edge of the coronary band on the left forelimb plus the thickened band of highly vascularised tissue that is located above the upper edge of the coronary band on the right forelimb)/2}.
Ratio of both eyes to both limbs	{(Region from the outer to inner canthus of the left eye plus region from the outer to inner canthus of the right eye)/2}/ {(The thickened band of highly vascularised tissue that is located above the upper edge of the coronary band on the left forelimb plus the thickened band of highly vascularised tissue that is located above the upper edge of the coronary band on the right forelimb)/2}.
Ratio of left to right eye	(Area that includes the orbit and periorbital area and runs from the left eye's outer to inner canthus)/(Area that includes the orbit and periorbital area and runs from the right eye's outer to inner canthus).
Behaviours in the forced lateralisation test	
Side of passing	In the lane, a cow passed an unfamiliar person to its left or right.
Standing	Taking longer than 5 s to stop and wait to pass, either to the left or right of the person in the lane.
Sniffing	Sniffing the ground, the individual, or a nearby cow in the lane.
Stopped while walking	Standing in any position in front of the person for fewer than 5 s.
Speed of walking in the FLT	Slow walking; more than 7 s for 5 m; medium walking; 4–7 s for 5 m; fast walking 3 s to cover 5 m when walking.
Tail movement	Tail moved quickly from its base around the hindquarters in a side-to-side flicking motion (raised tail) or hung freely from its body in a vertical line (relaxed tail).

Table 3
Mean infrared temperatures (IRT, °C) of eyes and limbs of lactating dairy cows, categorised as being left (L cow, n of P1= 78, P2= 55 and P3 =61) or right (R cow, n of P1 = 93, P2 = 116 and P3 = 110) side passing the person in lane, per period.

Variables	Period Number	L cows	R cows	SED[a]	P-value
IRT variables,°C					
Left eye	1	36.83	37.04	0.074	0.01
	2	37.03	37.05	0.072	0.82
	3	37.86	37.91	0.068	0.47
Right eye	1	36.83	37.03	0.079	0.002
	2	37.05	37.01	0.069	0.60
	3	37.88	37.92	0.056	0.59
Both eyes[b]	1	36.79	37.02	0.069	0.001
	2	37.04	37.03	0.065	0.91
	3	37.87	37.91	0.058	0.50
Left limb[c]	1	34.04	34.08	0.182	0.85
	2	33.14	32.82	0.211	0.15
	3	35.46	35.54	0.151	0.61
Right limb[c]	1	33.93	34.08	0.174	0.39
	2	32.94	32.61	0.216	0.16
	3	35.37	35.41	0.151	0.79
Both limbs[cd]	1	33.99	34.08	0.174	0.59
	2	33.04	32.72	0.203	0.13
	3	35.42	35.48	0.142	0.68
Ratio of both eyes to both limbs	1	1.08	1.09	0.005	0.47
	2	1.12	1.13	0.0067	0.12
	3	1.070	1.069	0.0037	0.86
Ratio of left to right eye	1	1.0023	1.0014	0.0018	0.60
	2	0.9995	1.0015	0.0015	0.22
	3	0.9993	0.9998	0.0011	0.67

[a] Standard error of the difference between two means is computed as (2*Adj Mean Square of Error/85.5), where 85.5 is the average number of animals in each period, which is equal to half of the total number of experimental animals (171).
[b] ((Maximum IRT of Left eye + Maximum IRT of Right eye) / 2).
[c] Coronary band of the forelimb.
[d] ((Maximum IRT of Coronary band of the left limb + Maximum IRT of Coronary band of the right limb)/2).

3.2. *Stepwise regressions of IRT variables as predicted by FLT behaviours and milk production variables*

The regression equations that explained the highest proportion of variance in IRT variables were in period 3, the lowest in period 1 (Table 5, Appendix 1). The regression equation for the IRT of both limbs had the highest proportion of variation explained (Adjusted $R^2 = 39.6$ %). The most consistent relationship was between IRT variables and waiting time before being milked. IRT of the left eye, right eye and both eyes were negatively related to waiting time before being milked in periods 2 and 3 ($P<0.01$) but not in period 1, while IRT of the left limb, right limb and both limbs were negatively related to waiting time before being milked in all 3 periods ($P<0.01$). The ratio of eyes IRT to limbs IRT was positively related to waiting time, emphasising the negative association between IRT of both limbs and waiting time ($P<0.01$) (Table 5, Appendix 1). Firstly, there was a negative relationship between the ratio of left to right eye IRT and sniffing (period 1), secondly, milk protein and slow walking (period 2). The majority of the IRT variables: left eye ($P<0.01$), right eye ($P<0.01$), both eyes ($P<0.01$), left limb ($P \geq 0.05 \leq 0.10$), right limb ($P \geq 0.01 <0.05$) and both limbs ($P \geq 0.01 <0.05$) were consistently related to laterality, but only in period 1, not in periods 2 and 3.

The number of parities was negatively related to the IRT of the left limb and both limbs in period 2, and with the left limb in period 1 ($P \geq 0.01 <0.05$). Age of animals was positively related to the left limb ($P \geq 0.05 \leq 0.10$) and both limbs IRT ($P \geq 0.01 <0.05$) in period 2, in period 1, it was negatively related to the right limb ($P \geq 0.01 <0.05$) and both limbs IRT ($P \geq 0.05 \leq 0.10$), and positively with the ratio of both eyes to both limbs IRT ($P \geq 0.01 <0.05$) (Table 5, Appendix 1).

Milk fat and protein content were negatively related to the IRT variables but in irregular patterns. For example, IRT of the left eye ($P \geq 0.05 \leq 0.10$) and left limb ($P \geq 0.01 <0.05$) were negatively related to milk fat content but only in period 3. Similarly, IRT of the right eye ($P<0.01$), both eyes ($P \geq 0.01 <0.05$) and both limbs ($P \geq 0.01 <0.05$) were negatively related to milk protein content in period 3, while the ratio of left to right eye's IRT ($P \geq 0.01 <0.05$) was negatively related to the milk protein content in period 2. In period 3, IRT variables: right eye ($P \geq 0.05 \leq 0.10$), both eyes ($P \geq 0.05 \leq 0.10$), right limbs ($P \geq 0.05 \leq 0.10$) and both limbs ($P \geq 0.01 <0.05$) were negatively related to milk secretion rate. Similarly, in period 1, the first speed of walking behaviour was negatively related to all eye IRT variables; left eye ($P \geq 0.01 <0.05$), right eye ($P \geq 0.01 <0.05$) and both eyes ($P<0.01$). In period 2, Relaxed tail behaviour was negatively related to the right ($P \geq 0.01 <0.05$) and both limbs ($P \geq 0.01 <0.05$) and positive to the ratio of both eyes to both limbs' IRT ($P \geq 0.01 <0.05$). Similarly, stopped while walking behaviour was negatively correlated with the right eye ($P \geq 0.01 <0.05$), right limb ($P<0.01$), and both limbs' IRT ($P<0.01$) and positively with the ratio of both eyes to both limbs' IRT ($P \geq 0.01 <0.05$). Sniffing behaviour was positively related to the right eye's IRT ($P \geq 0.01 <0.05$) and negatively with the ratio of left to right eye's IRT ($P \geq 0.01 <0.05$) in period 1 (Table 5, Appendix 1).

Daily milk yield was related to most IRT variables but in an inconsistent pattern. For example, in period 3, daily milk yield was positively related to both eyes ($P \geq 0.05 \leq 0.10$), left limb ($P \geq 0.01 <0.05$), right limb ($P \geq 0.01 <0.05$), and both limbs ($P \geq 0.01 <0.05$) and negatively with the ratio of both eyes to both limbs IRT ($P \geq 0.01 <0.05$). In contrast, in period 2, daily milk yield was negatively related to left ($P \geq$

Table 4
Milk productivity, and behaviours of lactating dairy cows, categorised as left (L cow, n of P1= 78, P2= 55 and P3 =61) or right (R cow, n of P1 = 93, P2 = 116 and P3 = 110) side passing the person in lane, for each of three periods.

Variables	Period	L cows	R cows	SED[a]	P-value
Productive variables					
Age (median[b]), months	1	53.7 (33, 38)	44.8 (46, 40)		0.38
	2	56.1(22, 28)	48.4 (56, 60)		0.30
	3	58.4 (22, 35)	46.0 (57, 43)		0.03
Time in milk, [d]	1	238.7	210.2	13.04	0.03
	2	275.6	248.7	13.5	0.07
	3	297.2	274.8	13.1	0.11
Daily milk yield, l/d	1	24.9	25.8	1.03	0.39
	2	22.0	22.5	0.84	0.58
	3	20.3	19.7	0.78	0.51
Somatic cell count (median), thousands/ mL milk	1	212.0 (30,46)	124.0 (55, 38)		0.01
	2	192.0 (20, 34)	116.0 (64, 50)		0.02
	3	121.0 (27,31)	97.0 (56, 51)		0.48
Milk fat content, g/ kg milk	1	31.5	29.6	1.39	0.19
	2	39.35	39.38	0.89	0.98
	3	40.7	39.6	1.40	0.45
Milk protein content, g/ kg milk	1	36.8	36.0	0.51	0.13
	2	35.4	35.3	0.40	0.76
	3	35.4	35.2	0.49	0.63
Milk secretion rate (median), mL/s	1	43.01 (45, 33)	45.6 (41, 52)		0.08
	2	41.1 (33, 21)	42.8 (52, 64)		0.05
	3	38.2 (34, 27)	40.7 (49, 55)		0.29
Behaviour variables, no. occurrences/d unless otherwise indicated					
Stopped while walking (median)[c]	1	2 (45, 31)	3 (57, 34)		0.40
	2	2 (37, 18)	2 (65, 51)		0.16
	3	1 (46,15)	1 (62, 48)		0.01
Sniffing (median)	1	2 (32, 46)	1 (60, 33)		0.002
	2	1 (29, 26)	1 (69, 47)		0.40
	3	1 (20, 14)	1 (53, 34)		0.83
Slow speed of walking (median)	1	1 (48, 29)	1 (58, 33)		0.02
	2	0 (30, 25)	0 (66, 49)		0.73
	3	1 (43,18)	2 (64, 46)		0.11
Medium speed of walking (median)	1	6 (47, 30)	6 (60, 31)		0.47
	2	4 (25, 30)	3 (61,55)		0.38
	3	3 (32, 29)	3 (66, 44)		0.34
Fast speed of walking (median)	1	0 (58, 20)	0 (70, 23)		0.89
	2	0 (50, 4)	0 (114,2)		0.06
	3	0 (53, 8)	0 (98,12)		0.68
Waiting time[d], min.	1	31.6	40.3	2.4	0.001
	2	38.65	38.72	2.7	0.98
	3	35.2	42.6	2.60	0.01
Relaxed tail (median)	1	4 (50, 27)	4 (63, 28)		0.01
	2	4 (31, 24)	4 (59, 57)		0.50
	3	6 (31, 30)	6 (67, 43)		0.20
Raised tail (median)	1	4 (47, 31)	4 (51, 40)		0.72
	2	5 (27, 28)	4 (69, 47)		0.20
	3	2 (44,17)	3 (48, 62)		<0.001

[a] Standard error of the difference between two means is computed as (2*Adj Mean Square of Error/85.5), where 85.5 is the average number of animals in each component, which is equal to half of the total number of experimental animals (171).
[b] The Mood's Median test was used to assess variables whose residuals were not normally distributed following logarithmic transformation. The first and second values within the bracket of a median indicate the number of cows with values less than or equal to the median and above the median, respectively.
[c] Stopping to stand for less than 5 seconds at any position in the lane.
[d] Before being milked, in the loafing area.

0.01 <0.05) and both limbs IRT ($P \geq 0.01 < 0.05$) and positive with the ratio of both eyes to both limbs IRT ($P<0.01$). Similar to period 2, in period 1, daily milk yield was negatively related to the right limb ($P \geq 0.05 \leq 0.10$), and both limbs' IRT ($P \geq 0.05 \leq 0.10$) and positively with the ratio of both eyes to both limbs' IRT ($P \geq 0.01 < 0.05$). Somatic cell count was positively related to the IRT of both eyes ($P \geq 0.05 \leq 0.10$) and both limbs ($P \geq 0.05 \leq 0.10$) in period 3 (Table 5, Appendix 1).

4. Discussion

Some evidence of connections between IRT and emotions were observed. Cows with higher IRT on their eyes were right lateralised, had more anxious behaviours such as the raised tails, higher waiting time in the milking yard before being milked, and lower milk fat and protein content. Most of these associations were not consistent across periods, being confined to period 1, which is probably due to the habituation of cows to the experimental setup and experimenter. However, altered situations such as threatening stimulus, ambient temperature and relative humidity cannot be ruled out. The negative relationship between limb IRT and waiting time in the yard before being milked was consistent across periods, which may indicate lower limb IRT as a measure of the efficacy of the cooling strategy for the herd.

4.1. The effects of laterality on IRT, milk productivity and behaviour variables

Right-side (R) passing cows had higher IRT in their eyes than that on the left side (L Cow). In our previous study, we also compared the effect of laterality on eye and limb IRT of cows (Uddin et al., 2019, 2021). Results were equivocal: Uddin et al. (2019) reported that R cows had higher IRT in their eyes and limbs than that of L cows, while Uddin et al. (2021) found no such association. Uddin et al. (2019) evaluated the two groups of extremely lateralised cows while Uddin et al. (2021) evaluated less than 50 randomised cows in 3 repeated periods. This study evaluated 171 randomly selected cows in 3 repeated periods where a relation between eye IRT and laterality has existed but no association with limb IRT was detected. This is probably because eye IRT indicates core body temperature while limb IRT indicates peripheral body temperatures (Cook et al., 2001; Byrne et al., 2017, 2019).

Negative emotions have been linked to elevated core body temperature in various species, including humans (Vinkers et al., 2013), mice (Borsini et al., 1989), cattle (Macaulay et al., 1995; Lee et al., 2018), and rats (Nakamori et al., 1993). It is likely that both the hypothalamo-pituitary adrenal axis and the autonomic nervous systems are involved, by increasing the basal metabolic rate. In dairy cows, injecting adrenocorticotropic hormone, corticotrophin-releasing hormone, and epinephrine can raise the maximum temperature of the eye (Stewart et al., 2007). Maximum IRT of the eye has also been linked to fear when handling cows and positively correlated with cortisol concentrations during the disbudding of calves (Stewart et al., 2008). The core body temperature is relatively consistent relative to peripheral body temperatures, which serve as a buffer between the internal body and the exterior ambient temperature (Sokabe and Tominaga, 2009). We discovered in our previous work (Uddin et al., 2021) that ocular IRT was more repeatable than that of limbs. Repeatable measurements are needed to track animals' emotional reactions over time, with habituation to the measure likely to be highly variable.

Cows with a right lateralisation are believed to be more nervous, in this study evidenced by less relaxed tails and more stopping whilst walking, as well as previously suggested by correlations with flight speed, crush score, and a faster walking pace (Phillips et al., 2015; Goma et al., 2018). In the study of Goma et al. (2018), anxiety was indicated by raised tails in dairy cows. Similarly, stressed sheep have demonstrated right side lateralisation (Robins et al., 2019).

The link with IRT is probably because the autonomic nervous system and hypothalamus pituitary adrenal axis becomes active in tense situations, which triggers the fight or flight response (Minton, 1994). Adrenaline tightens blood vessels, raising blood pressure, opening skin pores, and redistributing blood to the skin's surface (Minton, 1994). Due to the rich capillary beds of the posterior border of the eyelids and *caruncula lacrimalis*, which are highly innervated by the sympathetic nervous system and contain skin, hair follicles, sebaceous and sweat glands, and lacrimal tissue, these changes in blood flow result in increased heat emission from superficial capillaries around the eyes

Table 5
Directionalities of significant or close to significant correlations (α of 0.10 or less) between laterality and other behaviour indicators of stress or anxiety as the cows walked down a lane toward or past an unfamiliar person, and milk-related productivities, as factors in multiple regression models built to test the usefulness of maximum IRT measures of key external body surface regions in lactating dairy cows. The font of correlation values is in **bold** if P<0.01, italics if P ≥ 0.01 <0.05 and normal if P ≥ 0.05 ≤0.10.

Factors	Periods	Left eye	Right eye	Both eyes[a]	Left limb[c]	Right limb[c]	Both limbs[bc]	Both eyes/ both limbs[c]	Left eye/ right eye
Laterality[d]	1	- 0.18	- 0.25	- 0.20	- 0.21	*- 0.27*	*- 0.24*		
	2								
	3								
Age in month	1					*- 0.007*	- 0.006	*+ 0.00019*	
	2				+ 0.021		*+ 0.024*		
	3								
Parity	1				*- 0.094*				
	2				*- 0.42*		*- 0.43*		
	3								
Stopped while walking[e]	1								
	2		*- 0.039*			- 0.15	- 0.12	*+ 0.0040*	
	3								
Sniffing	1		*+ 0.061*						*- 0.0013*
	2								
	3								
Slow speed of walking	1		*- 0.062*	- 0.038					
	2								- 0.0012
	3								
Medium speed of walking	1								
	2								
	3				+ 0.069				
Fast speed of walking	1	*- 0.13*	*- 0.13*	- 0.14					
	2								
	3								
Waiting time[g]	1				- 0.031	- 0.026	- 0.028	+0.00096	
	2	- 0.008	- 0.010	- 0.0079	- 0.042	- 0.041	- 0.042	+ 0.0012	
	3	- 0.009	- 0.007	- 0.008	- 0.029	– 0.027	– 0.030	+ 0.00061	
Relaxed tail	1								
	2					*- 0.11*	*- 0.0995*	*+ 0.0035*	
	3								
Raised tail	1								
	2								
	3		+ 0.031						
Days in milk	1								
	2					- 0.065			
	3								
Daily milk yield	1					- 0.025	- 0.021	*+0.00081*	
	2				*- 0.049*		*- 0.048*	+ 0.0019	
	3			+ 0.015	*+ 0.043*	*+ 0.59*	*+ 0.052*	*- 0.00096*	
Somatic cell count	1								
	2								
	3			+ 0.00009			+ 0.00023		
Milk fat content	1								
	2								
	3	- 0.008			*- 0.019*				
Milk protein content	1								
	2								*- 0.00071*
	3		- 0.033	*- 0.010*			*- 0.06*		
Milk secretion rate	1								
	2								
	3		- 0.007	- 0.010		- 0.018	*– 0.021*		
Adjusted R^2 (%)	1	9.6	14.2	13.7	19.5	20.5	21.2	29.4	2.7
	2	7.1	14.1	10.6	31.7	29.5	35.3	26.1	4.7
	3	13.1	17.9	18.9	38.2	30.3	39.6	29.9	No terms

[a] ((Maximum IRT of Left eye + Maximum IRT of Right eye) / 2).
[b] Coronary band of the forelimb.
[c] ((Maximum IRT of Coronary band of the left limb + Maximum IRT of Coronary band of the right limb)/2).
[d] Log_{10} {(L+1)/(R+1)}, where L = number of left and R = number of right sides passing of cows, per day over 10 days.
[e] Stopping to stand for less than 5 s at any position in the lane.
[f] Before being milked, in the loafing area.

(Stewart et al., 2007, 2009; McGreevy et al., 2012). According to Breuer et al. (2000) and Hemsworth et al. (2000), cows exhibit their fear in the form of a quicker flight speed and decreased milk fat content when stock people frighten them with their behaviours. Similar to the findings of our earlier investigations (Uddin et al., 2019, 2021), R cows in this study exhibited higher milk secretion rates and reduced DIM, SCC, and age. Cows with lower DIM have, on average, had less experience being milked during that lactation and may be more sensitive to the milking system and any nearby hazards. Additionally, cows with a lower DIM could be anticipated to graze more actively in order to consume more feed to meet the demand for a high milk supply and a higher blood flow to support that. Compared to cows with more DIM, which would be more likely to be pregnant, cows with lower DIM may also be more likely to be in oestrus. The increased physical activity, vaginal blood flow, LH surge, ovulation, and progesterone release during the luteal phase that occur in oestrus cows are all likely to contribute to their increased emotional sensitivity (Redden et al., 1993; Talukder et al., 2015).

4.2. Association of IRT variables with a range of behaviours and milk production

The negative correlation between the limb IRTs and waiting time persisted across periods. As prospective indicators of the most at ease cows and those most likely to produce the most milk fat, a lower maximum IRT of the eyes and coronary band of the forelimbs and a higher waiting time prior to milking show promise (Uddin et al., 2019, 2021). Long waiting time allow cow to release more heat from their body parts such as limbs or to be cooled by sprinklers thus reduced limb's IRT. In contrast to a variety of other exterior body surfaces, the IRT of the limbs fluctuates significantly during the day in response to changes in feed intake (Montanholi et al., 2008). This is likely due to the extensive blood circulation that the muscular structure of the coronary band of limbs requires (Nikkhah et al., 2005; Alsaaod and Büscher, 2012). When faced with a new environment, such as an unexpected individual in their path, the coronary band in the limbs may experience changes in blood flow (Montanholi et al., 2008; Uddin et al., 2019). Additionally, the coronary band of the limbs works as a potential source of heat loss in cattle, according to reports from Klir and Heath (1992) and Van Den Heuvel et al. (2004). A cow that is emotionally calm (as indicated by greater waiting time and lower limb IRT) may secrete more oxytocin, which results in a more complete milk letdown process and higher milk fat content. Cows under stress are also known to limit their rumination, which aids in the digestion of forages (Osei-Amponsah et al., 2020). Stress responses increased adrenaline secretion, which in turn prevents the hypothalamus from secreting oxytocin. As a result, there would be less fat in the secreted milk and more residual milk with a high fat content in the udder as a result of the obstruction of milk let down. Goma et al. (2018) compared the milk fat percentages of cows that were behaviourally anxious (extremely right lateralised) and cows that were not anxious (extremely left lateralised), finding that the latter provided milk with a lower fat percentage. In contrast, cattle with a calm temperament measured slower flight speed were considered to have a "good" temperament. Cattle with more excitable temperaments, as measured by faster flight speed, chute score, pen score, and exit velocity, exhibit greater basal concentrations of glucocorticoids and catecholamines (Burrow and Dillon, 1997; Petherick et al., 2002). In Australia, breeding initiatives have been encouraged to use cattle with good temperaments due to their increased output (Burrow and Corbet, 2000). The fact that milk fat and protein content were inversely correlated with the IRT factors in this investigation supports the idea that stressed cows produce milk of poor quality. The daily milk production data from this study reveals a variable relationship between IRT variables and milk fat and protein content. Some IRT variables were positively and some negatively related to milk fat and protein content, which is probably reflecting the fact that as the amount of milk varies more liquid milk typically has less milk fat and or protein content. A short waiting time and low milk fat content may have contributed to a high level of residual milk fat in the udder due to insufficient milk letdown and clearance from the udder. Given that somatic cell counts have been associated with stress, the association between limb IRT and somatic cell counts may once again point to cows that are more tense (Nasr and El-Tarabany, 2017; Malik et al., 2018).

4.3. IRT and laterality

In this sample of randomly selected cows, IRT variables had a positive relationship with right laterality in period 1. This suggested that body surface IRT increased in correlation with cows' levels of anxiety. Contrary to our prior work (Uddin et al., 2019) and other studies (Goma et al., 2018; Lee et al., 2018; Phillips et al., 2015), there was no association between IRT measurements and the laterality index in period 2 and 3. This may reflect the fact that many of the cows had been used previously for forced lateralisation tests and habituation to the presence of the person may have occurred more rapidly than with naïve cows. In the study of Uddin et al. (2019), cows' IRT of their eyes and limbs was positively correlated with the right laterality. This was explained by the fact that when a cow passes someone (posing a threat) on their right side, the cow sees the person with their left eye, which is primarily connected to the right brain hemisphere. It is here that the fight or flight centres are located and activated during the events that cause anxiety (Bristow and Holmes, 2007; Phillips et al., 2015; Du et al., 2018; Goma et al., 2018). According to Lee et al. (2018), pharmacologically induced anxious beef calves have higher rectal temperatures, as well as more attentive and vigilant behaviours compared to non-anxious cattle. The IRT response to anxiety may operate over a shorter time horizon than the laterality index, which is a more permanent measure, according to our earlier discovery that the laterality index is a highly reproducible, long-term metric (Goma et al., 2018). The design of laterality studies may influence the ability to detect differences, because it is best evaluated on populations of animals that have distinctly different emotions. Two distinctly different cow groups were chosen for the studies by Uddin et al. (2019), Goma et al. (2018), and Phillips et al. (2015) based on their emotional states (as measured by left- and right-side lateralisation). Similar to this, Lee et al. (2018) utilised anxious and non-anxious beef cattle that had been provoked by medication. Contrarily, in the current study, cows were chosen based on milk output ranges and DIM rather than on their unique and various emotional states. Alternately, it's possible that IRT and laterality are detecting various animal emotions. Sniffing and the left-to-right eye IRT ratio had a negative association, which shows that the IRT disparities between the two eyes were biologically significant, at least in Period 1. The inverse correlation with sniffing, which was linked with R cows in this study and that of Goma et al. (2018), and left eye dominance, which is logically anticipated to be associated with an elevated temperature, support this. The tendency towards a negative correlation between the left to right eye ratio and slow walking was also anticipated because R cows in the study by Goma et al. (2018) also showed more slow walking. However, it must be noted that although these were both significant or close to significant relationships ($P = 0.02$ and 0.09, respectively), the R^2 values were low and there was no correlation with the FLT. The significant negative relationship with milk protein content suggests that if the ratio is of biologically relevant it may have correlations to production variables.

To conclude, the results support our projected hypothesis in the first period of the study. Eye IRT had a positive association with the behavioural responses of negative emotions such as right laterality, while the association was negative between eye IRT and milk quality (milk fat and protein content). The negative relationship between the maximum IRT of limbs and waiting time was consistent across periods.

Further research is recommended before automated non-invasive IRT technologies can be applied on-farm. The forced lateralisation test also requires further validation as a predictor of level of anxiety, for example via evaluations in pharmacologically induced stressed or anxious compared to relaxed cows. Strong negative emotional stimulants may be necessary to evoke changes in IRT associated with changes in emotions. It is also advised to use cows with similar DIM and SCC, in a similar environmental THI.

Declaration of Competing Interest

Authors declare that there were no conflicts of interest.

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Appendix

Table. 1
Multiple regression models were created to test the utility of IRT measures of key external body surface regions, their combination, and ratios (Y Variables) in lactating dairy cows. Equations showing significant correlations (of 0.10 or less) between laterality and other behaviour indicators of stress or anxiety as the cows walked down a lane towards or past an unfamiliar person, and milk related productivities (X Variables), were used as factors in the models.

Y variable of stepwise regression	Periods	X variables of Step wise regression
Left eye	1	36.98 (±0.041) - 0.18 (±0.052, P = 0.001) Log_{10}((L+1)/(R+1)) - 0.13 (±0.050, P = 0.01) Fast speed of walking **(Adjusted R^2 = 9.6 %).**
	2	37.3 (±0.091) - 0.0075 (±0.0021, P = 0.001) Waiting time **(Adjusted R^2 = 7.1 %).**
	3	38.6 (±0.19) - 0.009 (±0.002, P < 0.001) Waiting time - 0.008 (±0.004, P = 0.06) Milk fat content **(Adjusted R^2 = 13.1 %).**
Right eye	1	36.9 (±0.066) - 0.25 (±0.055, P < 0.001) Log_{10}((L+1)/(R+1)) + 0.061 (±0.027, P = 0.03) Sniffing - 0.062 (±0.026, P = 0.02) Slow speed of walking - 0.13 (±0.053, P = 0.01) Fast speed of walking **(Adjusted R^2 = 14.2 %).**
	2	37.5 (±0.104) - 0.010 (±0.0020, P < 0.001) Waiting time - 0.039 (±0.019, P = 0.04) Stopped while walking **(Adjusted R^2 = 14.1 %).**
	3	39.6 (±0.47) - 0.007 (±0.002, P < 0.001) Waiting time - 0.007 (±0.004, P = 0.05) Milk secretion rate - 0.033 (±0.011, P = 0.003) Milk protein content + 0.031 (±0.02, P = 0.05) Raised tail **(Adjusted R^2 = 17.9 %).**
Both eyes	1	37.0 (±0.052) - 0.20 (±0.048, P < 0.001) Log_{10}((L+1)/(R+1)) - 0.038 (±0.022, P = 0.09) Slow speed of walking - 0.14 (±0.047, P = 0.003) Fast speed of walking **(Adjusted R^2 = 13.7 %).**
	2	37.3 (±0.078) - 0.0079 (±0.0018, P < 0.001) Waiting time **(Adjusted R^2 = 10.6 %).**
	3	39.4 (±0.56) - 0.008 (±0.002, P < 0.001) Waiting time + 0.015 (±0.009, P = 0.09) Daily milk yield - 0.010 (±0.004, P = 0.01) Milk secretion rate + 0.00009 (±0.00005, P = 0.09) Somatic cell count - 0.010 (±0.004, P = 0.01) Milk protein content **(Adjusted R^2 = 18.9 %).**
Left limb	1	35.4 (±0.24) - 0.21 (±0.12, P = 0.095) Log_{10}((L+1)/(R+1)) - 0.094 (±0.047, P = 0.05) Parity - 0.031 (±0.0052, P < 0.001) Waiting time **(Adjusted R^2 = 19.5 %).**
	2	35.5 (±0.57) + 0.021 (±0.011, P = 0.06) Age in month - 0.042 (±0.0055, P < 0.001) Waiting time - 0.42 (±0.16, P = 0.01) Parity - 0.049 (±0.019, P = 0.01) Daily milk yield **(Adjusted R^2 = 31.7 %).**
	3	36.5 (±0.51) - 0.029 (±0.004, P < 0.001) Waiting time + 0.043 (±0.017, P = 0.01) Daily milk yield - 0.019 (±0.008, P = 0.02) Milk fat content + 0.069 (±0.041, P = 0.097) Medium speed of walking **(Adjusted R^2 = 38.2 %).**
Right limb	1	35.9 (±0.37) - 0.27 (±0.12, P = 0.03) Log_{10}((L+1)/(R+1)) - 0.007 (±0.003, P = 0.03) Age in month - 0.025 (±0.013, P = 0.06) Daily milk yield - 0.026 (±0.005, P < 0.001) Waiting time **(Adjusted R^2 = 20.5 %).**
	2	36.6 (±0.56) - 0.041 (±0.006, P < 0.001) Waiting time - 0.065 (±0.019, P = 0.001) Daily milk yield - 0.15 (±0.06, P = 0.02) Stopped while walking - 0.11 (±0.046, P = 0.01) Relaxed tail **(Adjusted R^2 = 29.5 %).**
	3	36.2 (±0.47) – 0.027 (±0.004, P< 0.001) Waiting time + 0.59 (±0.020, P = 0.01) Daily milk yield - 0.018 (±0.009, P = 0.05) Milk secretion rate **(Adjusted R^2 = 30.3 %).**
Both limbs	1	35.9 (±0.37) - 0.24 (±0.12, P = 0.04) Log_{10}((L+1)/(R+1)) - 0.006 (±0.0031, P = 0.05) Age in month - 0.021 (±0.013, P = 0.09) Daily milk yield - 0.028 (±0.005, P < 0.001) Waiting time **(Adjusted R^2 = 21.2 %).**
	2	35.98 (±0.58) + 0.024 (±0.010, P = 0.02) Age in month - 0.042 (±0.005, P < 0.001) Waiting time - 0.43 (±0.16, P = 0.01) Parity - 0.048 (±0.018, P = 0.01) Daily milk yield - 0.12 (±0.056, P < 0.001) Stopped while walking - 0.0995 (±0.042, P = 0.02) Relaxed tail **(Adjusted R^2 = 35.3 %).**
	3	38.6 (±1.18) – 0.030 (±0.004, P < 0.001) Waiting time + 0.052 (±0.019, P = 0.01) Daily milk yield – 0.021 (±0.009, P = 0.02) Milk secretion rate + 0.00023(± 0.00011, P = 0.05) SCC- 0.06 (± 0.03, P = 0.03) Milk protein content **(Adjusted R^2 = 39.6 %).**
Ratio of both eyes to both limbs	1	1.019 (±0.010) + 0.00019 (±0.00009, P = 0.03) Age in month + 0.00081 (±0.00036, P = 0.03) Daily milk yield +0.00096 (±0.00014, P < 0.001) Waiting time **(Adjusted R^2 = 29.4 %).**
	2	1.018 (±0.018) + 0.0012 (±0.00019, P < 0.001) Waiting time + 0.0019 (±0.00061, P = 0.003) Daily milk yield + 0.0040 (±0.0020, P = 0.04) Stopped while walking + 0.0035 (±0.0015, P = 0.04) Relaxed tail **(Adjusted R^2 = 26.1 %).**
	3	1.06 (±0.010) + 0.00061 (±0.0001, P < 0.001) Waiting time - 0.00096 (±0.0004, P = 0.03) Daily milk yield **(Adjusted R^2 = 29.9%).**
Ratio of left to right eye	1	1.004 (±0.0012) −0.0013 (±0.00056, P = 0.02) Sniffing **(Adjusted R^2= 2.7 %).**
	2	1.03 (±0.011) - 0.00071 (±0.00030, P = 0.02) Milk protein content - 0.0012 (±0.00067, P = 0.09) Slow speed of walking **(Adjusted R^2 = 4.7 %).**
	3	There are no terms in the model.

Where, Log_{10} {(L+1)/(R+1)} = Log_{10} laterality where L = number of left and R = number of right sides passing of cow, per day over 10 days; in a period.

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