Interpretative summary

Lateralisation of dairy cow behaviour responses to conspecifics and novel persons, Phillips.

Increased left eye use has been observed in cattle when viewing threats. We examined lateralised eye use in cows responding to other cows and novel and familiar operators. Dominant cows were less likely than subordinates to use their left eye to monitor confrontations with other cows. Cows predominantly using their left eye in interactions with other cows were more likely to view an unfamiliar person in the centre of a track with their left eye by passing the person on the right, and they had higher crush restraint scores, compared with cows predominantly using their right eye. Left eye use appears more common in response to threats in dairy cows.

RUNNING TITLE: LATERALISATION OF BEHAVIOUR IN DAIRY COWS

# Lateralisation of behaviour in dairy cows in response to conspecifics and novel persons

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## ABSTRACT

The right brain hemisphere, connected to the left eye, co-ordinates fight and flight behaviours in a wide variety of vertebrate species. We investigated whether left eye vision predominates in dairy cows' interactions with other cows and humans, and whether dominance status affected the extent of visual lateralisation. Although there was no overall lateralisation of eye use to view other cows during interactions, cows that were submissive in an interaction were more likely to use their left eye to view a dominant animal. Both subordinate and older cows were more likely to use their left eye to view other cattle during interactions. Cattle that predominantly used their left eye during aggressive interactions were more likely to use their left eye to view a person in unfamiliar clothing in the middle of a track by passing them on the right side. However, a person in familiar clothing was viewed predominately with the cows' right eye when they passed mainly on the left side. Cows predominantly using their left eyes in cow to cow interactions showed more overt responses to restraint in a crush compared to cows who predominantly used their right eyes during interactions (crush scores: left eye users 7.9, right eye users 6.4, SED = 0.72, P = 0.01). Thus interactions between 2 cows and between cows and people were visually lateralised, with losing and subordinate cows being more likely to use their left eye to view winning and dominant cattle and unfamiliar humans.

**Key words:** dairy cow; dominance; hemispheric processing; visual lateralization

## INTRODUCTION

Lateralisation occurs when one hemisphere of the brain controls the cognitive processing of a specific situation and is manifested as a contra-lateral side bias, such as handedness (Rogers, 2000; Schönweisner et al., 2007; Richter et al., 2009). Lateralisation is widespread among vertebrates (Basile et al., 2009) and describes those behaviors, including motor, sensory, and cognitive responses, that are consistently biased to one side of the body at either the individual or population levels (Baraud et al., 2008; Robins and Phillips, 2010; Komárková and Bartošová, 2013). It is thought that lateralisation functions to facilitate multitasking through different tasks being processed in different hemispheres (Güntürkün et al., 2000; Rogers, 2000; Rogers et al., 2004; Dharmaretnam and Rogers, 2005; Ghirlanda et al., 2009) and to aid social communication and predator avoidance (Vallortigara et al., 2010). A better understanding of lateralisation in cows may assist in understanding the emotions they experience and what stimuli they perceive to be threatening and stressful.

Ungulates are good candidates for highly lateralized vision, since the extremely lateralised location of their eyes allows them to scan for predators within two monocular fields, united in a broad field of vision of approximately 330°, with a blind spot only directly behind them (Piggins and Phillips, 1996). Ruminants orientate towards their object of vision by turning their head rather than their pupil (Piggins and Phillips, 1996). The high degree of decussation of bovine optic nerves in the optic chiasm (Herron et al., 1978) allows sensory cues and information coming from the left visual field to be analyzed in the right cerebral hemisphere and vice versa (Baraud, et al., 2008). The right hemisphere is specialized in both perceiving and expressing emotions and serves the function of responding to unexpected stimuli, controlling escape functions, and detecting and responding to predators, especially from the left side (Komárková and Bartošová 2013; Robins and Phillips, 2010; Rogers, 2010). The left eye/right hemisphere specialization for spatial processing in novel or exploratory contexts can be related to broader vigilance functions (Robins and Phillips, 2010). Horses showing preferential left-eye use (indicating dominance of the right brain hemispheres) show increased fear and aggression compared to those with dominant left hemispheres (Komárková and Bartošová, 2013). The left hemisphere controls an individual's response to food items and analysis of recalled cues in cattle (Robins and Phillips, 2010), and well-established patterns of behavior performed in non-stressful situations in a wide range of species (Rogers, 2010). Left hemisphere specialisation and dominance is most likely in animals not expressing fear or aggression (Komárková and Bartošová, 2013).

Cattle exhibit hierarchical organisation within the herd, and the resulting dominance order may reduce aggression and stress within the herd. As stressed animals rely on predominant use of the right hemisphere (Rogers, 2010), lateralisation of eye use could be an indicator of stress susceptibility. A link between dominance and another lateralised behaviour, persistency of lateralised milking parlour entry, has been found previously (Prelle at al., 2004).

Some animals also display  bilateral behavioural asymmetry, with behaviors involving one of two opposing limbs (e.g. initiation of walking) performed more on either the right or left side of the body, demonstrating a difference in preference or ability between the two sides (Annett, 1985). Such laterality may also be expressed by parts of the body extending sideways during routine behaviors, e.g. tongue movement when eating. Such behavioural laterality may be related to asymmetry in body morphology. For example, diagonal symmetry of bovine hooves probably derives from asymmetrical walking or lying patterns (Phillips et al., 1996). Lateralized walking in cattle has also been demonstrated as side preferences in a

T-maze (Arave et al., 1992), during entry to a milking parlor (Paranhos da Costa and Broom, 2001) and lying (Uhrbrook, 1969; Arave and Walters, 1980; Bao and Giller, 1991). Laterality may also occur because internal body parts are not symmetrical, e.g. the foetus is positioned towards the right side of the body, explaining left side laterality during lying in pregnant (Wilson et al., 1999), ruminating (Albright and Arave, 1997) cows.

Cattle prefer to view a novel person in their left eye (Robins and Phillips, 2010), suggesting that they view that person as a potential predator. It is not clear whether similar visual lateralisation might be present in cow to cow interactions, especially in the case of a subordinate cow engaged in an agonistic encounter with a dominant cow. It is conceivable that a person would be viewed by all cows as a dominant leader of the herd (Albright, 1986), whereas most cows will dominate some of their herdmates. Dominance is in part dependent on temperament (Kramer et al, 2013), and there is increasing evidence that differences in the degree of lateralisation are associated with temperament in a variety of species, such as dogs (Branson and Rogers, 2006; Batt et al., 2009), horses (McGreevy and Thomson, 2006) and humans (DeYoung et al., 2010).

We hypothesized that the social context of cow:cow or cow:people interactions would influence predominant eye use, and that subordinate cows, those in losing encounters and those showing fearful temperament traits may demonstrate greater use of their left eye than right eye during agonistic encounters with conspecifics and novel encounters with humans, as a result of signal processing in the right hemisphere of the brain. We further anticipated that the response to a human might depend on whether that person appeared familiar or not. In addition we investigated relationships between eye use laterality and their temperament, as well as their productivity characteristics that may relate to priority of access to feed resources.

## MATERIALS AND METHODS

The study utilised the dairy herd of the University of Queensland at Gatton, comprising 183 Friesian cows and 50 cows with mixed breed status, based on Friesian crosses with Jersey, Brown Swiss and Angus. Mean milk yield, body condition score (Lowman et al., 1976) and age ($\pm$ standard error) of cows in the herd were 25.5 $\pm$ 0.47 l/d; 3.2 $\pm$ 0.277 and 4.8 $\pm$ 0.13 years, respectively. At 1700 h, after pm milking, cows were turned out into a feedlot, where they were offered a total mixed ration at two 60 m feed bunks (providing 52 cm trough space per cow), with two 5 m water troughs at one end bunks (providing 4.3 cm trough space per cow), separated by a central concrete passage. At 0500 h cows were brought in for am milking and afterwards, at 0700 h, they were sent out to pasture, from which they returned for pm milking at 1445 h.

### *Study 1. Cow behaviour in the feedlot and milking parlour*

All 233 cows, identified from their ear tags, were observed engaging in agonistic interactions at the feed bunk, in the feedlot and in the field. Preliminary observations determined that most agonistic behavior occurred after milking from 0700 to 0900 h, and from 1130 to 1330 h. All cows were observed by a single recorder (HO) during these times for 25 d, spread over a 5 wk period. During each interaction, each cow was classified as being in one 6 possible positions (Figure 1). To determine the subordinate/dominant status of the cows, 3 subordinate behaviours were recorded, any one of which was assumed to indicate that cows had lost the interaction: *a*, moved body away from other animal; *b*, moved head away from other animal, and *c*, no movement. Two dominant behaviours were recorded, either of which was assumed to indicate that the cow had won: *d*, touched the other animal with head, or *e*, moved head towards other animal. During each interaction it was noted which eye they predominantly used to look at the other cow during the interaction, as determined by the orientation of their face.

***Study 2. Forced lateralised movement tests***

Observations were made of lateralisation of walking down a track and possible correlations between this track behaviour and visual lateralization and individual dominance values. Track walking lateralisation was chosen because in the absence of any disturbance in the track, such as a person located there, cows show a normal distribution of this behavior, whereas most other behaviours demonstrate a bimodal distribution (Phillips et al., 2003). A total of 169 and 138 cows were observed in two studies, with individual identification by ear tags and freeze brands. Cows were observed after pm milking, after taking a step down from the concrete surrounding the milking parlour, walking down a 5 m wide earth track, bordered by two lines of metal fencing and without any worn routes on either side (Figure 2). A novel person stood approximately 10 m down the track and facing the cows, thus forcing the cows to pass on the left or right side of the track. In study 1 the person was dressed in familiar blue overalls, as normally worn by the veterinary students that worked regularly with the cows in this herd; but in study 2 the person wore green overalls, a face mask, hat and glasses to present a novel person stimulus. In study 1, on alternate days there was either the person was positioned on the track or not, in order to assess lateralisation of passing a familiar person when compared with a control group without the person. When no person was present on the track, cow laterality was assessed by someone hidden in an adjacent crush.

The entire herd of cows were observed for the number of right and left side passes (from the cows' perspective), indicating viewing of the person predominantly in the left and right eye fields of vision, respectively. In study 1, the side each cow passed the person was recorded a mean of 5 times/cow on 11 individual d over a period of 21 d, alternating daily between the person being in the crush and on the track. Scores of left or right side were awarded as the cow walked past the person, and on days that the person was absent an additional middle score was included when no side preference was obvious. The records of 15 cows that missed some days and 47 cows that did not have pre-recorded dominance values were omitted from the analysis. Side changes during passage down the track were recorded but were too rare to allow statistical analysis.

***Study 3. Tests of cow temperament***

Eight predominantly right and 8 predominantly left eye using cows were selected from records of their interactions with other cows in Study 1 (ratios of <1 and >3 for left:right eye use ratios, respectively, with the method of calculation detailed in statistical analysis below) and right and left lane use in the second study of part 2. Temperament testing was carried out in these cows using a crush test to assess response to restraint in the presence of a human and an open field test to assess response to social isolation in a novel environment. One cow was removed from each group due to poor health and an extreme response to the crush test, which threatened the cow's welfare. Tests were carried out between 0800 h and 1200 h and were repeated 3 times for each cow.

The crush score was used to assess the degree of restlessness of each cow, based on a categorical rating scale that assessed a) willingness to enter the crush, b) willingness to place their head in the head bail, c) movement and respiration type in the crush over a 2 min period, and d) additional scores for kicks, vocalisation, kneeling and lying attempts (Kilgour et al 2006). Total scores were created by addition of a – d. Following exit from the crush, Flight

Speed was recorded as the time taken for each cow to cover a distance of 2m, using the front feet as reference points (Petherick et al 2002).

The open field test was used to examine cows' coping responses to physical, visual and social isolation and a novel environment (Kilgour et al 2006). A bare earth collecting yard of 6 x 9 m with 1.6 m high solid sides and a non-slip floor was established at least 20 m from the rest of the herd. The floor of the yard was divided into 6 equal-sized squares by spray paint. Over a 5 min period an observer recorded the behaviour of the cattle from small slits in the solid sides, thus avoiding observer influences on cattle behaviours. The behaviours recorded were: a) number of squares entered (defined as both front feet placed in the square); b) number of escape attempts performed, such as pushing at the sides; c) number of vocalisations; d) number of defecations and urinations. Faecal contamination of the yard was removed between test subjects.

***Statistical analysis***

***Study 1.*** To investigate the significance of left/right eye use in winning and losing cows the ratio of total number of times cows used their left eye (LE) to the total number of times that they used their right eye (RE) was calculated, with the addition of 1 to LE and RE data to avoid any zero value numerators or denominators, which would give zero or infinity ratio values, respectively. The ratio for cows when they won was regressed against the ratio for cows when they lost. The dominance value of each cow was determined from the records of its interactions with other cows, using the method of Clutton-Brock et al. (1979), which incorporates information on the dominance status of animals interacted with. We chose this method over others because it is robust for large datasets with a high proportion of cows that do not interact with each other (Bang et al., 2010). The formula for calculating the dominance value of each animal was as follows:

$$DV = (B + \Sigma b + 1)/(L + \Sigma L + 1)$$

Where:

DV = dominance value

B = number of cows beaten

$\Sigma b$ = total number of cows that the beaten cows beat, excluding the subject

L = number of cows that it lost to

$\Sigma l$ = total number of cows which the winning cows lost to, excluding the subject

The significance of differences in position adopted in cow-cow interactions and behaviour for winners and losers was explored by Pearson's chi-square tests. Linear regression was used to obtain further information on significant correlations, after testing for non-linear characteristics of the fitted line and the distribution structure of the residuals by the Anderson Darling test.

***Study 2***. Data were expressed as the proportion of cows walking down the left hand side of the track. In study 1, d 1 data was discarded because only 23 out of 165 cows were able to be identified from the observation position within the crush on that day. Chi squared analyses were used to assess the significance of deviation from an equal left and right passage.

***Study 3.*** General Linear Models were constructed to investigate the differences between the selected RE and LE cows in crush score, flight speed and the open field test, as well as mean milk yield, days in milk, lactation number, and dominance value. Residuals were tested for normal distribution using the Anderson-Darling test. Crush score and open field test total had normally distributed residuals but flight speed did not, and data distribution was not improved by transformations, therefore a Mood's median test was used for this variable. Pearson correlation coefficients were calculated to assess the relationships between days in milk, average daily milk yield, lactation number, LE:RE ratio, left/right ratio (from cow to cow interactions), crush score, flight speed, open field score, track left/right ratio and dominance value.

The statistical package Minitab (version 16) was used for all calculations, with results considered significant if $P < 0.05$.

## RESULTS

### *Study 1. Cow behaviour in the feedlot and milking parlour*

A total of 992 interactions were recorded, and the distribution by position and behaviour are shown in Table 1. Of these, 25 losing and 43 winning cows were excluded from analysis because none of the pre-determined behaviours were exhibited. Twelve cows showed some head to head with bodies aligned and head to head with bodies at 180° behaviours but were judged to be losers because of more extreme movements by the second cow. The most frequent position adopted was head to head with bodies aligned, and then head to side. The most frequent behaviour for losing cows was no movement, then moving their body away and then moving their head away. Nearly all of the behaviours of winning cows comprised a head swing towards the other animal.

Over all cows and interactions, there was no significant difference in eye use during the interactions (mean number of times eye used/cow: LE 2.12, RE 2.12, SED 1.01, $P = 0.92$). However, there was a significant positive relationship between the LE:RE ratio for winning cows with that for the losing cows (Figure 3):

$$\text{LE:RE ratio}_{\text{winning cows}} = 0.66 \text{ (SE 0.0483) x LE:RE ratio}_{\text{losing cows}} \quad (P < 0.001)$$

where LE:RE ratio is (LE + 1)/(RE+1)

This positive relationship indicates that cows that used their left eye more than their right eye when winning also did the same when losing. A coefficient of 1 would indicate that the relationship between use of left and right eyes was exactly the same for winning and losing cows. However, as the coefficient was less than unity (0.66), cows showed less extreme left eye laterality when winning than losing, indicating that there was a reduced chance that these winning cows would use their left eye than losing cows. There was a tendency for there to be a higher ratio of left to right eye use when the cows demonstrated behaviour *c*, no movement, than behaviours *a* or *b*, moving their body or head away (LE:RE means *a* 1.14; *b* 1.13; *c* 1.25; SED 0.0634, $P = 0.10$).

Dominance values were not normally distributed (Anderson Darling test $P < 0.005$), but $\log_{10}$ transformed DV values were (Anderson Darling test, $P = 0.27$) (Figure 4). There was a negative relationship ($P = 0.01$) between the ratio of left eye to right eye use in the losing cows and $\log_{10}$ transformed dominance values (dv):

$$\text{LE:RE ratio}_{\text{losing cows}} = -0.39\ (\pm\ 0.148)\ \log_{10} \text{dv}$$

Hence the more dominant a losing cow was, the greater the likelihood that she would use her right eye in interactions. Similarly in the winning cows there was also a negative relationship ($P = 0.01$) between the ratio of left eye to right eye use and transformed dominance values (dv), indicating that the more dominant winning cows were the more likely they were to use their right eye:

$$\text{LE:RE ratio}_{\text{winning cows}} = -0.38\ (\pm 0.147)\ \log_{10} \text{dv}$$

Hence dominance is more influential than winning or losing in determining eye use. There was a positive correlation between dominance value and age of the cows (Spearman Rank Correlation Coefficient 0.39, $P < 0.001$), and a positive relationship between age and the ratio of left eye use to right eye use in both losing and winning cows:

Losing cows: $\text{LE:RE ratio}_{\text{losing cows}} = 6.4 \times 10^{-3}\ (\pm 3.46 \times 10^{-3})$ age; ($P < 0.001$)

Winning cows: $\text{LE:RE ratio}_{\text{winning cows}} = 6.1 \times 10^{-3}\ (\pm 3.84 \times 10^{-3})$ age; ($P < 0.001$)

Thus both winning and losing cows were more likely to use their left eye as they aged. There was also a positive relationship between body condition score and use of the right eye by winning cows (Spearman Rank Correlation Coefficient 0.15, $P = 0.05$).

***Study 2. Forced lateralised movement tests***

***Part A. Experimenter in familiar clothing.*** In the first set of measurements of cow track walking behavior, 14.7% of the cows took a middle path, which was consistent over time and results are therefore presented only for cows going left or right. The majority, 70-90%, of the cows initially walked down the left side viewing the experimenter in their right eye when the experimenter in familiar clothing was present, and they maintained this for the remainder of the measurements, except for d 18 (Figure 5). When the experimenter was present in the crush, the majority of cows (84%) walked down the right side in initially, but over the next 4 d this progressively changed to the left side, until over 90% walked down the left side.

***Part B Experimenter in unfamiliar clothing.*** When the experimenter wore unfamiliar clothing, a mask and hat and stood in the center of the track, most cows walked to the right of the track, viewing the experimenter in their left eye, on the first day ($\chi$ = 29.7, P = 0.01)(Figure 6). After this they walked down both sides equally, except that on d 3 and 9 cows again walked to more to the right side ($\chi$ = 17.5 and 5.5, respectively, P = 0.05), which appeared to be as a result of disturbances during milking on that day.

The histogram of left and right side passage down the track shows a bimodal pattern, with most cows walking consistently down the left or the right side of the experimenter over the 14 d (Figure 7).

***Study 3. Tests of cows' temperament***

There was an increase in right:left side ratio of passage down the track for LE cows, compared with RE cows ($\log_{10}$ values: LE 0.85, RE -0.82 [antilog 7.1, 0.15, respectively, SED 0.156, P <0.01). LE cows had greater total crush scores than RE cows (LE 7.9, RE 6.4, SED = 0.72, P = 0.01), and the crush score was correlated with Dominance Value (CC = 0.67, P = 0.009). There was no difference in total open field test scores for LE and RE cows (LE 20.3, RE 16.0, SED = 5.88, P = 0.41), but there was correlation between the open field test score and dominance value (CC 0.68, P = 0.001) and a tendency for crush score to be correlated with open field test score (CC 0.48, P = 0.068). Crush score was negatively correlated with LE:RE ratio (CC -0.67, $P = 0.02$) and correlated to left to right side ratio in Part B of the track study (correlation coefficient 0.59, $P = 0.02$).

There were no significant differences in individual components of the scores, except that there was a significant increase in escape score of LE cows in the open field test (squared values: LE 31.5, RE 8.8, SED = 24.69, $P = 0.03$). However, residuals were not normally distributed ($P < 0.005$) and some caution is warranted. There was no significant difference in flight speed between eye groups (LE 0.47, RE 0.49, SED $P = 0.98$). There was a positive correlation between LE:RE ratio in the between-cow confrontations and the left to right side ratio in Part B of the track study (correlation coefficient 0.68, $P< 0.01$).

## DISCUSSION

### ***Study 1. Cow behaviour in the feedlot and milking parlour***

The results of this study demonstrate that the contest-losing cows and subordinate cows were more likely to use their left eye in encounters, thus suggesting that their flight or fight response was heightened. We accept that there may have been an element of chance involved in the orientation of the two cows, especially in the more crowded locations, such as the milking parlour. However, this is not likely to have introduced any systematic bias into the measurements, but may have increased the random variation in orientation measurements, which we overcame with relatively large numbers of animals for the study.

In conditions of restricted resources, social hierarchy functions to limit aggression. Despite this, in dairy cow systems subordinate dairy cows are the subject of regular aggression from dominant cows (Castro et al., 2011), causing them to experience more stress during encounters. In this study we recorded approximately 10 interactions per hour of study, or 0.04 interactions per cow per hour, demonstrating relatively high rates of aggression. There is limited evidence that subordinate cows are more nervous than dominant cows, with lower productivity levels and potentially less efficient digestive behaviour (Reinhardt, 1973; Phillips and Rind, 2002). They are willing to sacrifice food quality to avoid contact with dominant cattle (Rioja-Long et al., 2012), and the positive association of body condition score with right eye use in the present study would support this.  We investigated body condition score as a potentially useful proxy measure for dominance, knowing that there was potential for error surrounding the measurement of the latter as a result of the large number of cow:cow interactions that need to be measured. However, the statistical evidence for relations between eye use and dominance was actually stronger than between dominance and body condition score, which suggests that our measurement of dominance was robust.

At a physiological level, androgen treatment of cattle enhances their dominance and reduces fearfulness (Boissy and Bouissou, 1994). The relationship between social dominance of cattle and their temperament is therefore complex and probably context specific. One study with beef cattle has suggested that middle ranking cattle have least stress (la Lama et al., 2013), but others found no relationship (Partida et al, 2007). Lateralisation of parlour entry has, however, been detected more in dominant than subordinate cows (Prelle at al. (2004). Studies are needed in dairy cows to relate social dominance to temperament and stress-related behaviour.

***Study 2*. *Forced lateralised movement tests***

Cows appeared to initially respond differently to the person in familiar clothing and unfamiliar clothing. Familiar clothing led to left side passage, viewing the person in their right eye, which was consistent over time, whereas on the first day the unfamiliar person was largely passed down the right side, viewing the person on their left side. This agrees with research by Robins and Phillips (2010) in which the first passage across a novel person's path when bisecting the herd was predominantly from right to left, viewing the person in their left eye, and the second passage bisecting the herd after it had settled was in the opposite direction, viewing the person in their right eye. The response to the person in the crush, initially to the right, viewing the person in the left eye, and then increasingly to the left over time, viewing the person the right eye, may indicate a fear response to the presence of the person initially, which is quickly attenuated.

There was a strong correlation between ratio of left to right eye use in the cow to cow confrontations and the left to right ratio in the track study; this indicates that cows consistently had a preferred eye to view all interactions they encounter. Cows clearly had a preferred eye to view novel stimuli (person in track) and they were relatively consistent in how they viewed this stimulus.

***Study 3*. *Tests of cows' temperament***

Cows that predominantly used their left eye in cow to cow interactions and cow to human interactions had higher total crush scores. A greater crush score describes an animal with a restless disposition when a person is present, and the link to left eye use provides further evidence of a heightened flight or fight response in these cows (Robins and Phillips, 2010). There was also a correlation between crush and open field scores, which was to be expected as they are both measures of activity responses to a stressful situation. The absence of any difference between predominantly RE and LE cows in the open field test may be due to a variety of factors. One is the small sample size in this study, and although the number of escape attempts was significantly greater in LE cows the residuals were not normally distributed. A larger sample size might have overcome this problem. The open field test is a test commonly used for dairy cows, with movement, vocalisations, time spent immobile and exploration time being the most repeatable measurements (Forkman et al., 2007). However, there is concern about which emotions it measures: in heifers locomotion is more related to activity than fear of novelty (Boissy and Boissou, 1995), and in calves it is more related to the social isolation (de Passille et al., 1995). As our cows were group-reared, it is likely that social isolation was the biggest factor affecting scores in the open field test (Munksgaard and Simonsen, 1996). Inactivity could indicate a settled nature but alternatively may suggest that the cow is stressed by separation from conspecifics (Boissy and Boissou, 1995), making interpretation of the test results difficult. As a test of fear responses, the open field test correlates to some degree with behaviour seen in other fear tests (Boissy et al., 1995, Kilgour et al., 2006), but the correlations are not strong (Forkman et al., 2007), hence we used it in combination with other tests.

This study found that dominance value was positively correlated with crush and open field scores. This suggests that dominant cows are more disturbed by the presence of a person (crush score) and novel environment (open field test) than subordinate cows, whereas their increased use of the right eye in interactions with other cows suggests that they are less fearful in the presence of conspecifics. This is expected because the dominance value was measured from interactions with other cows in Study 1. In this study there was no correlation between LE:RE and dominance values, however in Study 1 dominant cows were more likely to use their right eye in interactions with losing cows, thus showing that their response to fearful stimuli was less aroused than in subordinate cows.

## CONCLUSIONS

Losing and subordinate cows were more likely to use their left than right eye to view the other cows during interactions. This suggests a heightened flight or fight response. The forced lateralisation test described appears to be a suitable test to explore the emotional responses of cattle to novel stimuli. Cows that predominantly used their left eye in all types of interactions had a more fearful temperament as indicated by heightened response to confinement to the crush, indicating that these individuals perceived this as more stressful. We conclude that the eye that dairy cows use in interactions with other cattle and humans can provide valuable information on their temperament.

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**Table 1**

Recorded movements of losing (Pearson Chi-Square = 1417, DF = 70, P < 0.001) and winning cows (Pearson Chi-Square = 1586, DF = 70, P < 0.001). Position codes: A, head to head, bodies at 180$^{o}$ ; B, head to head, bodies at right angles; C, head to side; D, head to head, bodies aligned; E, head to tail, bodies at 180$^{o}$; F, head to tail, bodies aligned. Behaviour codes: *a*, moved body away from other animal; *b*, moved head away from other animal, or *c*, no movement; *d*, touched the other animal with head, and *e*, moved head towards other animal.

| | *Behaviour* | | | | | |
|---|---|---|---|---|---|---|
| Position | *a* | *b* | *c* | *d* | *e* | Total |
| Losing cows | | | | | | |
| A | 30 | 7 | 5 | 0 | 1 | 43 |
| B | 12 | 1 | 5 | 0 | 0 | 18 |
| C | 71 | 5 | 15 | 2 | 0 | 93 |
| D | 245 | 103 | 445 | 0 | 9 | 802 |
| E | 3 | 0 | 6 | 0 | 0 | 9 |
| F | 1 | 0 | 0 | 0 | 0 | 1 |
| Total | 362 | 116 | 476 | 2 | 10 | 966 |
| | | | | | | |
| Winning cows | | | | | | |
| A | 2 | 0 | 2 | 0 | 29 | 33 |
| B | 0 | 0 | 1 | 0 | 14 | 15 |
| C | 1 | 0 | 2 | 0 | 89 | 92 |
| D | 10 | 0 | 19 | 0 | 770 | 799 |
| E | 2 | 0 | 0 | 0 | 6 | 8 |
| F | 1 | 0 | 0 | 0 | 0 | 1 |
| Total | 16 | 0 | 24 | 0 | 908 | 948 |

**Table 2**

The ratio of left to right eye use (with addition of 1) for losers and winners in aggressive interactions

| Variable | Mean | SE Mean | Minimum | Median | Maximum |
|---|---|---|---|---|---|
| Left loser | 2.15 | 0.124 | 0 | 2 | 10 |
| Right loser | 2.10 | 0.126 | 0 | 2 | 13 |
| Left winner | 2.10 | 0.163 | 0 | 1 | 11 |
| Right winner | 2.14 | 0.171 | 0 | 1 | 17 |

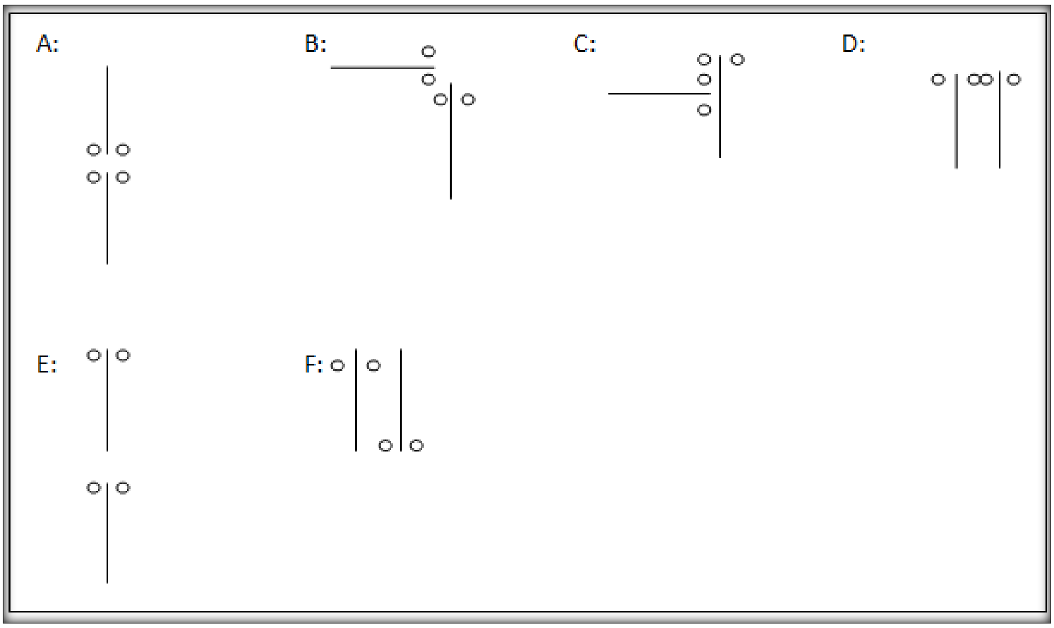


Figure 1. Diagramatic representation of the body positions of cows during interactions. A: head to head, bodies at 180$^{o}$ ; B: head to head, bodies at right angles; C: head to side; D: Head to head, bodies aligned; E: head to tail, bodies at 180$^{o}$; F: head to tail, bodies aligned

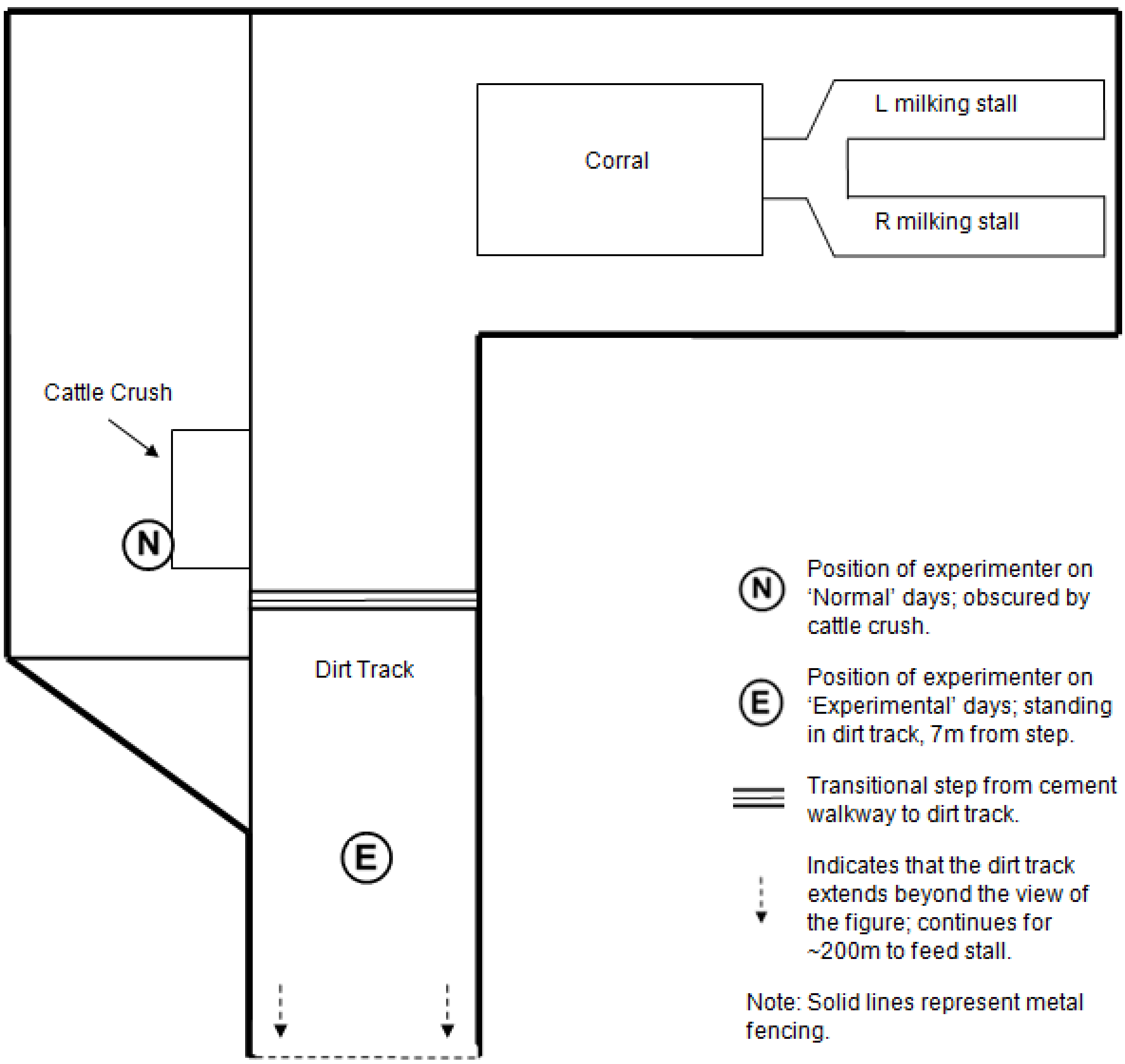


Figure 2 Milking parlour and track leading from it in which the cows were observed

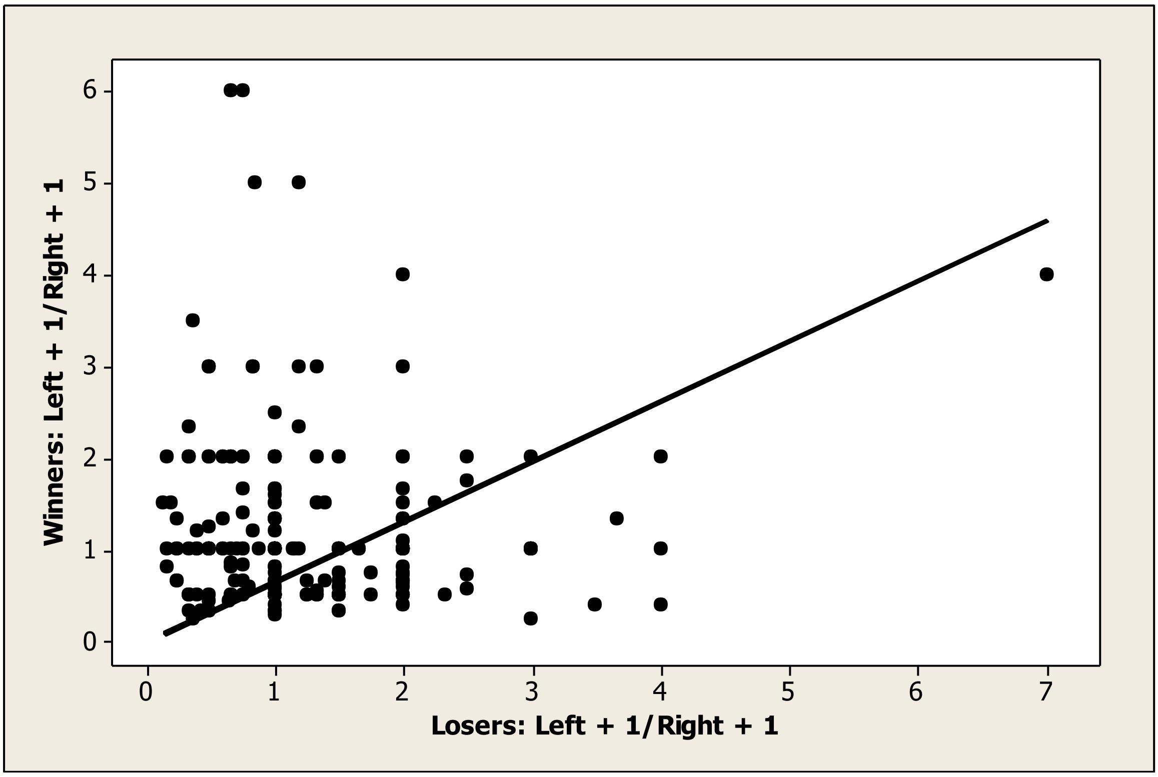


Figure 3. Relationship between winners and losers in their ratio of left to right eye use

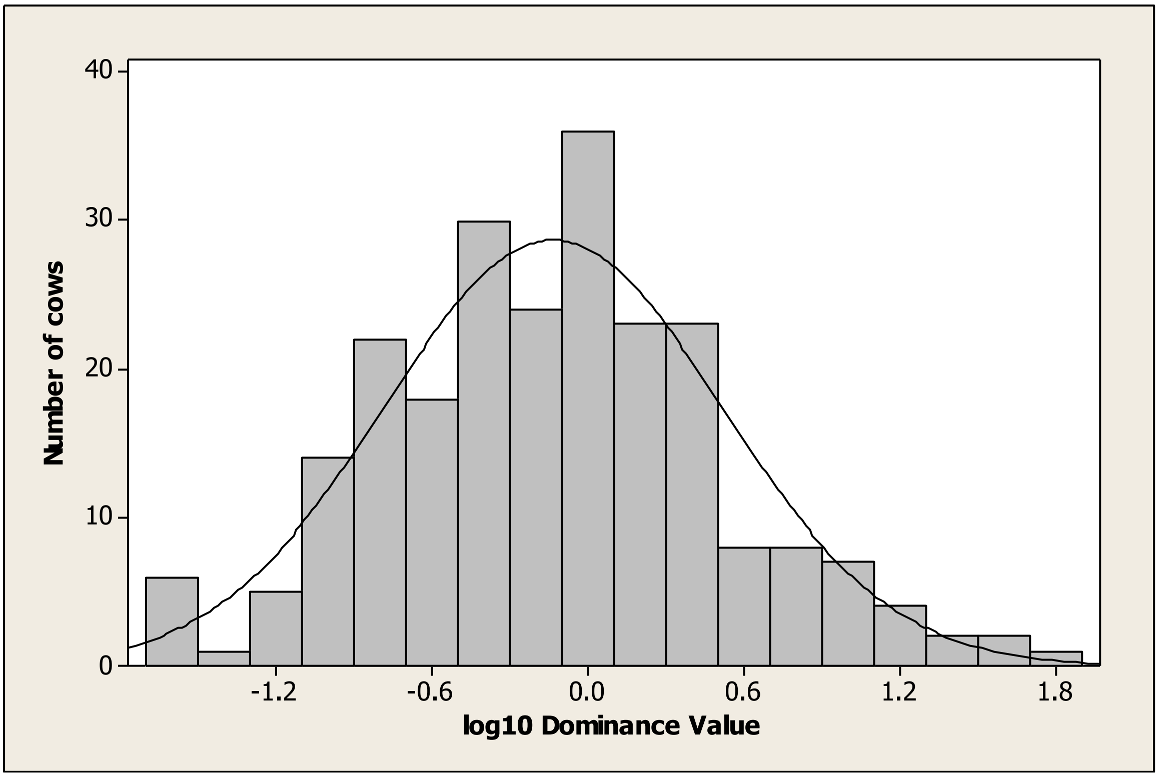


Figure 4 Distribution of $Log_{10}$ Dominance Values, with normal distribution curve

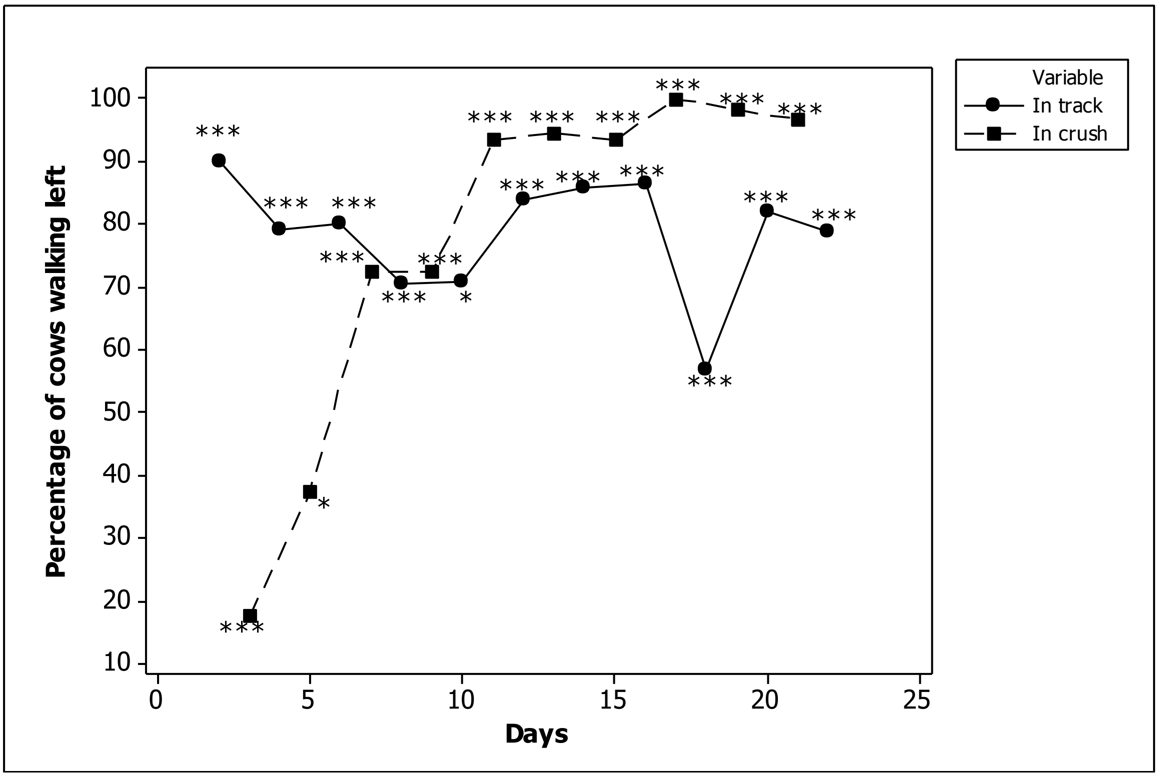


Figure 5. Percentage of cows walking down the left side of the track with the experimenter in familiar clothing either in the track or in the crush. * P ≤ 0.05; *** P ≤ 0.001

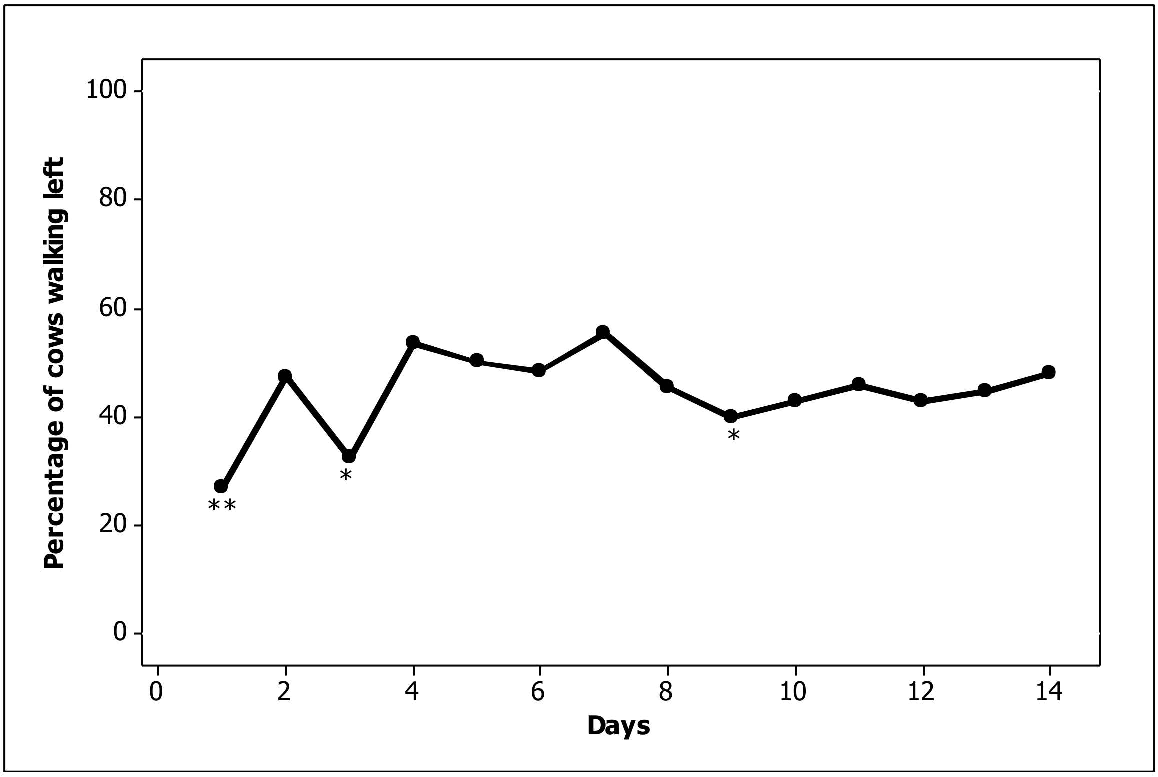


Figure 6. Percentage of cows walking down the left side of the track with the experimenter in unfamiliar clothing and wearing mask in the track. * P ≤ 0.05

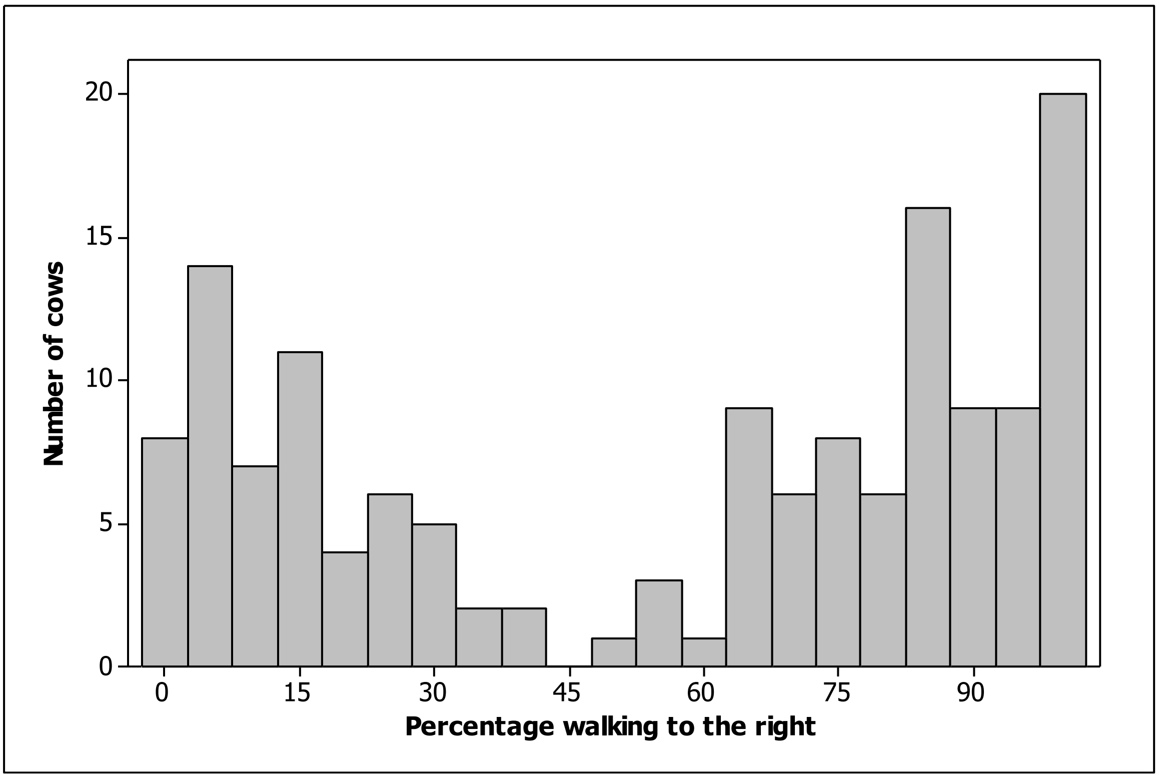


Figure 7 Proportion of cows (n = 148) walking to the left side of the experimenter over 14 d