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Is the response to humans consistent over productive life in dairy cows?

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Abstract

Dairy cattle have a high level of interaction with humans throughout their productive life. Welfare and productivity are affected if cows find these interactions aversive, so tests assessing fear of humans have been included in welfare assessment protocols. Practicality issues suggest that all animals on large farms cannot be tested. If a sub-sample is chosen, then animal factors affecting the response must be investigated. To assess the effect of age, 114 Holstein cows were tested at regular intervals across their productive lifetime. Animals were tested at 12-15 months of age, first breeding, prior to first calving, then at early, mid and late lactation for 1st and 2nd lactations and into their 3rd lactation. The test involved approaching each cow when standing in the passageway of the barn with sufficient space to retreat. Response was recorded on a 0-8 incremental scale, and several qualitative terms were scored using sliding scales from absence to full presence. There was a significant effect of age on response. Cows became more approachable with increasing age, up until the middle of the first lactation, with no further change beyond this stage. Cows became more at ease and less nervous with increasing age. Individual cow within-group rankings for tests at each stage showed correlation with rankings in the following stage. As this is a single-farm study, further research is necessary to assess interaction of factors such as housing, breed and quality of human handling on the long-term development of fear of humans. However, the results suggest that the age of the animal tested affects the response, and that animals of different age groups should be tested when a sub-sampling is required to assess welfare on large farms.

Keywords: animal welfare, dairy cow, fear, human approach, temperament, welfare assessment

Introduction

In most dairy farming systems, frequent contact with humans is a necessary part of the daily routine for lactating cows. Because of this, it is desirable that the relationship is a positive one from both the animal's and the farmer's point of view (eg Waiblinger *et al* 2006). The quality of the interaction is known to affect the welfare and productivity of the cows. Fearfulness of handlers reduces milk yield (Rushen *et al* 1999; Breuer *et al* 2000), and cows that are handled in a forceful or negative manner are more reluctant to approach a human experimenter (Breuer *et al* 2000; Hemsworth *et al* 2000) and avoid human contact (Waiblinger *et al* 2003). Cows can recognise individual handlers and learn to avoid or approach them accordingly (Munksgaard *et al* 2001).

Due to the importance of the quality of the human-animal relationship, it is desirable to include a measure of fearfulness of humans in on-farm welfare assessment tools. A number of welfare assessment protocols for dairy cattle (including Welfare Quality® [2009]) use an avoidance test as a measure of fear of humans. Producers, consumers and

other stakeholders will have confidence in the welfareassessment protocols if the farm-level results are reliable. The reliability aspect of the human approach/avoidance tests at the cow level has been the subject of a number of studies. The response of individual cows to an approaching human shows consistency when tested repeatedly on a single day or over a period of weeks (Windschnurer *et al* 2008; Gibbons *et al* 2009) and consistency at the farm level when revisited on a bi-monthly basis (Winckler *et al* 2007) and have been shown to correlate well with other tests of fear of humans (Rousing & Waiblinger 2004; Gibbons *et al* 2011).

A problem with implementing comprehensive welfare assessment protocols on farms with large numbers of animals is the time, and consequently, cost, of performing the assessment. This has led to sampling strategies being investigated, in which not all of the animals are assessed (eg Main *et al* 2010). For the sampled group to reliably represent the whole farm population, the factors affecting the measure must be understood so that they can be taken into account when choosing the appropriate individuals to use in the subsample. The age of the animal is probably one of these

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variation systematic sources of in human approach/avoidance response. Response to milking in primiparous heifers declines over time in both handled and nonhandled animals (Hemsworth et al 1989). It is possible that response to human approach also declines with age. However, Waiblinger et al (2003) found no consistent relationship between age and human approach, with some animals becoming more fearful of humans with age on some farms, and other less so. No data on ages were given, but the differences may be due to differences in quality of handling or breed on these farms. To understand how responses to human approach might develop and change over time, a study on an experimental farm where individual animal's responses can be followed over time would be informative. The aim of this project was to assess the development in response of dairy cattle in an experimental herd to a human approach test (HAT) from prior to entry to the main lactating herd and through the major productive stages of their lives.

Materials and methods

Study animals, management and test schedule

The study was conducted at SAC's Dairy Research Centre in Dumfries, UK. A total of 114 Holstein heifers born and reared on the farm were selected for the study. The animals were part of a herd of 200 lactating cows that was managed and monitored as part of a long-term genotype by farming system study. There were two genotypes ('Select' and 'Control') balanced across two farming systems. The 'Select' group had been selected over many generations for high milk solids, while the 'Control' group were selected to represent the UK rolling average for milk solids. After first calving, animals from each genotype were assigned to either a low forage (LF) diet and kept indoors throughout the year or to a high forage (HF) diet with grazing during the summer. Prior to first service, all maiden heifers were housed with access to cubicles with mattresses and sawdust covering, in one of two management pens: the 'pre-breeding pen' for the younger animals and the 'breeding' pen for those being mated. The heifers had ad libitum access to a total mixed ration (TMR) consisting of 69% grass silage and 31% concentrate on a fresh weight basis. Free access to water was provided. The heifers were served using AI when they reached a weight greater than 330 kg, at approximately 15 months of age, with the aim of first calving at 24 months of age. After pregnancy was confirmed, heifers were turned out to pasture during the spring and summer period (April to September) or kept in a cubicle shed during the winter period (October to March) until 2-3 weeks before calving when they were moved to the calving shed. After first calving, the animals were moved to the main lactating herd and allocated to system (HF or LF). Lactating cows were milked three times per day (0400, 1300 and 2000h), and housed with access to one cubicle per cow with mattresses and sawdust covering. The farm staff on the farm remained the same throughout the trial. The handling quality was not recorded formally as part of this trial, but was observed as being positive and quiet when assessed during training for other welfare assessment projects (eg Rutherford et al 2009).

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A human approach test (HAT) was carried out on each animal at several time-points during the first five years of life, hereafter referred to as productive life stage (ProdLifeStage). The first HAT was performed in the 'prebreeding pen' when heifers were between 12 and 15 months of age. The second test was made the 'breeding pen' when the animals were between 16 and 17 months of age, at the time of first service. In practice, the heifers did not spend very long in the pre-breeding pen and were often moved to the breeding pen earlier than 16 months, so most received their first test in the breeding pen. A total of two tests were made in the heifer barn to assess repeatability of the measure. The repeatability results are reported in Gibbons et al 2011. The third test was made 2 to 3 weeks prior to first calving. Tests were then made early (30-50 days in milk [DIM]), in the middle (130-150 DIM) and late (230-250 DIM) in each of the following lactations. All animals were allowed to settle into their new social groups for at least a week before being tested. Most dairy cows were tested throughout their first lactation, 50% entered their second lactation with a few cows being tested in their third lactation.

Human approach test

The human approach test (HAT) was the same as used in Gibbons et al (2009), but is briefly described here. To allow the animal to express a full avoidance or approach response, each animal was approached while standing in a passageway with sufficient clear space (2 m) around to move away. The experimenter approached diagonally from the front towards the cow's neck, starting from a distance of 3 m, moving in steps of approximately 1 m, pausing for 10 s after each step. The cow was considered to have withdrawn when she made two or more steps away from the experimenter. The response of the cow was assessed using a rating scale and using qualitative terms. The response was scored on a 0 to 8 scale according to the distance at which she began to move away and the level of physical interaction she allowed. The resulting score is referred to as the ResponseScore. The levels for the ResponseScore are shown in Table 1. The qualitative aspects were also scored by rating the response for nine qualitative terms ('at ease', 'nervous', 'interested', 'friendly', aggressive', 'shy', 'bold', 'fearful' and 'docile') using a 125-mm visual analogue scale, with a rating of 0 indicating that the aspect of expression was entirely absent, and a mark of 125 mm indicating that expressive quality was entirely present throughout the test. The qualitative scores allowed the response to be better described and interpreted. The tests were carried out by five trained experimenters. One of the experimenters did 67% of the tests across all years. The experimenters were trained by the most experienced experimenter to familiarise them with the technique. Intra-observer reliability tests indicated high levels of concordance between the five observers (Spearman's rank correlations of over 80% for all, except for 'docile' between Observer 1 and 5 which was 75%).

Statistical methods

During testing, it seemed that some of the qualitative terms were highly correlated and quantifying positive and negative aspects of the same characteristics of the animals' demeanour. The relationships between the qualitative variables were assessed with a Principal Components Analysis (PCA) to determine if some of the terms were redundant and whether a smaller subset would adequately represent all ten terms. The sums of squares and products method was used as the variables were continuous and all measured on the same scale.

As the ResponseScore was a categorical trait, a GLMM analysis with a binomial distribution and a logistic link function was fitted. Inspection of the residuals suggested that the scores for the qualitative terms were normally distributed. REML was used to assess the scores for the qualitative terms that were identified as being the most informative from the PCA described above. Models were constructed for each independent variable using a forward stepwise technique. Each independent variable was firstly tested in a univariate analysis using animal identity as a random model, and became a candidate for the multivariable model only if it had a *P*-value less than 0.25. Factors were fitted in the multivariable model according to their Wald statistic. Potential fixed and random effects were tested for their effects. ProdLifeStage, test season (summer, autumn, winter, spring), age at test (in number of quarter years), observer, location (indoors or outdoors), sire, genetic group (control and select) and feed group (HF and LF) were tested. Age at test was excluded as it was confounded with ProdLifeStage, but was of lower statistical significance. Sire identity also significantly affected ResponseScore and a number of the qualitative terms, which may indicate that there is an underlying heritability to the trait. However, for purposes of this analysis, the sire identity was used in the random model, with animal identity nested within sire, and observer as an additional random effect. Only fixed effects that were significant in the final model are presented in the results.

Non-parametric Spearman's rank correlations were used to examine the correlation between the ResponseScores at each ProdLifeStage. As the majority of the animals were first tested in the breeding pen, this ProdLifeStage was used as the start-point.

Results

In the course of the experiment, 114 animals were tested. Some animals were lost from the experiment each year due to sale, culling or death. No animal was culled for 'poor temperament' (the farm's description of being difficult to handle or milk). The number of animals tested at each ProdLifeStage is presented in Table 2. There were too few animals in midlactation 3 to be considered further in the analysis.

The first two dimensions of the PCA for the qualitative variables explained 77 and 7% of the variation, respectively. A number of terms loaded on opposite ends of Dimension 1, but with similar scores for Dimension 2, indicating that a number of them were likely to be assessing the same aspects of the cow's demeanour. The terms 'shy', 'fearful' and

Table ITable showing ordinal scale used to score the cow'sresponse to the approaching experimenter in the HAT.

Score	Response
0	Cow moves away before experimenter reaches 3 m
I	Cow moves away when experimenter is 3-2 m away
2	Cow moves away when experimenter is 2–1 m away
3	Cow moves away when experimenter is > 1 m away
4	Cow moves away when experimenter is 0 m away
5	Cow moves away as experimenter extends arm to touch
6	Cow moves away as experimenter touches the cow's head/shoulder
7	Cow does not move away when touched on head/shoulder
8	Cow approaches/walks towards experimenter

Table 2The number of animals tested in eachProdLifeStage.

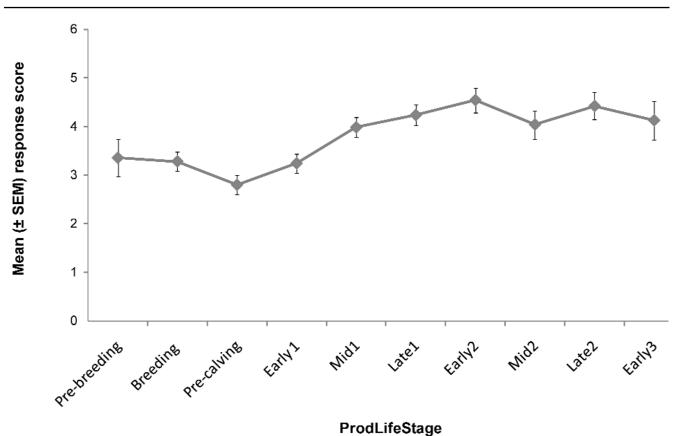
ProdLife Stage	Number of animals				
Pre-breeding pen	41				
Breeding pen	114				
Pre-calving	88				
Early lactation I	104				
Mid lactation I	105				
Late lactation 1	98				
Early lactation 2	59				
Mid lactation 2	50				
Late lactation 2	38				
Early lactation 3	8				
Mid lactation 3	3				

'nervous' all loaded positively on Dimension 1, while 'interested', 'bold' and 'friendly' loaded negatively. All of these terms loaded negatively on Dimension 2, while 'at ease' and 'docile' loaded positively. 'Aggressive' had loadings of close to zero on both dimensions, probably due to a high number of 'entirely absent' scores. Terms with the highest loadings were chosen to represent the two dimensions. The term 'nervous' was chosen to represent Dimension 1. This term had highly negative scores for Dimension 2, so was contrasted with 'at ease' which had a high positive score and represented Dimension 2.

There was a significant effect of ProdLifeStage on ResponseScore (Wald = 63.65; $F_9 = 7.07$; P < 0.001) indicating that animals became more approachable with time (Figure 1). There was also a significant effect of ProdLifeStage on the ratings for the qualitative terms. Animals were scored as generally becoming less nervous (Wald = 63.32, $F_9 = 7.04$; P < 0.001) and more at ease (Wald = 54.92, $F_9 = 6.10$; P < 0.001) with increasing age. There was no effect of location of test (indoors or outdoors), test season, feed group or genetic group (P > 0.05 for all).

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Graph showing mean ResponseScore across all ProdLifeStages. A higher response score indicates that the experimenter was able to get closer to the cow than a lower score. Raw data and standard error bars are shown.

Table 3	Spearman	's rank correlation	s and significance	e levels for Res	sponseScore for	each pair of Pro	odLifeStages.
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ProdLifeStage	Breeding pen	Pre-calving	Earlyl	Midl	Latel	Early2	Mid2	Late2
Breeding pen	-	0.472 [†]	0.245	0.439†	0.403	0.348	0.258	0.041
Pre-calving			0.43 l†	0.202	0.436 [†]	0.176	-0.134	-0.316
Earlyl				0.341	0.452 [†]	0.207	0.490*	0.274
Midl					0.774***	0.711**	0.433 [†]	0.345
Latel						0.753***	0.442 [†]	0.366
Early2							0.324	0.394
Mid2								0.587*
Late2								

*** P < 0.001; ** P < 0.01; * P < 0.05; † P < 0.10.

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The data shown in Figure 1 suggested that ResponseScore stabilised from the middle of the first lactation onwards. To examine this, the ProdLifeStages were divided into two categories: 'young' (Early1 and previous) and 'old' (Mid1 and beyond), and the analysis re-run with this age category as a factor. There was a significant difference in ResponseScore between the 'old' and 'young' categories (Wald = 35.59, $F_1 = 35.59$; P < 0.001; 'young' = 3.2 and 'old' = 4.0). As a further test of stability, the effect of ProdLifeStage on ResponseScore was tested within the 'old' life-stages only. No effect of ProdLifeStage was detected (Wald = 8.25, $F_5 = 1.65$; P = 0.147), indicating that the response was now stable. Table 3 shows the correlations between tests. For six out of eight stages there was a significant relationship or a tendency for a relationship with the next stage, but relationships beyond this are irregular.

Discussion

The results show that the Holstein dairy cows on this farm became more approachable up until the middle of the first lactation, when the response stabilised. The cows became less nervous and more at ease with the approach of a human with time. It is possible that the increase in the ResponseScore was due to the repetition of the test procedure. However, the final scores for the Lactation 3 cattle who had received up to eleven tests were not dissimilar to those reported for multiparous cattle tested once only (data from Gibbons et al 2009). Location of test, test season and genetic group did not affect the response, which may indicate robustness in the test procedure. It appears that repeated experience with humans over many consecutive handling and milking episodes reduced the cows' fear of humans. This suggests that they did not find the experience of handling or milking aversive, as cows become less willing to approach humans when they have previously found the experience aversive (Rushen et al 1999; Hemsworth et al 2000; Munksgaard et al 2001).

However, this study documents a single population of cattle. In a study of a number of breeds on multiple farms, Waiblinger et al (2003) found no consistent relationship between age and response to humans. Further research is needed to understand the effects of breed, housing and management on the fear of humans (eg Mazurek et al 2011) to add to what we know already about the effects of poor handling. However, both studies demonstrate that age, or more pertinently, duration of experience of human handling and milking, affects the response to a human approach or avoidance. While good consistency in human avoidance test results has been shown when farms are revisited or animals retested (eg Waiblinger et al 2003; Winckler et al 2007; Windschnurer et al 2008), these studies were done on farms where the size of the herd meant it was possible to re-test all or almost all of the cows. The average dairy farm in Scotland and Denmark has in excess of 110 animals (DairyCo 2011; Danish Cattle Federation 2011) and over 380 in New Zealand (DairyNZ 2011). With herds of these sizes, welfare assessments can only be made practical by testing a subsample of the herd. Selecting animals at random for testing

may mean that results are not consistent between consecutive visits, even if underlying fear of humans does not change. This may reduce farmer confidence in the assessment protocol. Using appropriate criteria to select a representative sample is important to achieve reliability, and age may be one of these criteria. Ideally, some animals from a number of age categories (perhaps including youngstock) should be tested to give a complete picture.

Many of the ResponseScores at the different stages of productive life showed significant correlation with scores at the following one. This suggests that the trait is reasonably stable over a few months, but is affected by the individual's experience with humans or other environmental factors over longer periods. Very few studies have assessed a behavioural trait over a period of more than a few months, and within the reproductive cycle to this extent. A number of capture: recapture studies in wild animals have shown a correlation between temperament between years (Reale *et al* 2000; Gabriel & Black 2010), indicating that behavioural traits may be stable over time.

Animal welfare implications

Welfare assessment protocols have the potential to substantially increase animal welfare on commercial farms by providing consumers with information on animal welfare. Levels of fearfulness of humans are an important consideration. If a sub-sample of animals must be selected for testing in large herds, then age of cow must be taken into account in selection. It may be ideal to test some younger and some older animals.

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