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On-farm testing of dairy calves' avoidance response to human approach: Effects of sex, age and test order

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Abstract

Human approach tests are generally accepted as valid measures of the human-animal relationship and hence are widely included in on-farm welfare assessment protocols. Most measures of avoidance response to human approach in production animals have been developed and tested under experimental conditions rather than on commercial farms, thereby making the results less relevant for operational on-farm animal welfare assessment. By contrast, the current study was conducted on calves in their home pens. On 110 Norwegian dairy farms, 548 group-housed calves (aged 22–288 days) were tested individually for their behavioural response to an unfamiliar human approach by a single test person. To conduct the test, the respective calf manager administered concentrates to the manger, followed by the test person who approached each animal in turn in a standardised manner. The avoidance response of the individual calf was categorised as 0 to 5 (maximal to no avoidance) in reaction to an attempted approach and head touch by the test person. The statistical analyses showed that heifer calves were more avoidant compared to bull calves, as were younger bulls compared to older bulls, and that overall avoidance increased in calves that were not tested first.

Keywords: *animal welfare, avoidance response, dairy calves, fear, human-animal relationship, human approach test*

Introduction

Tests of avoidance response in animals to human approach are generally accepted as valid measures of the human-animal relationship (HAR) (Waiblinger *et al* 2006) and are widely included in on-farm welfare assessment protocols (eg Welfare Quality® [Winckler *et al* 2009]). The animals' reactions to humans can result from a number of different emotions, including fear (Waiblinger *et al* 2006). Fear and anxiety are undesirable in production animals, and prolonged exposure can have a profound impact on welfare and production (eg Rushen *et al* 1999a; Smulders & Algers 2009). Fear can be transferred across different situations and contexts (Lecorps *et al* 2018a), making the animals hard to approach and increasing their flight distance (Breuer *et al* 2003). Fearful animals can be more difficult and dangerous to handle (Rushen *et al* 1999b) and recent research has shown that fearfulness is highly consistent within individual animals over time (Lecorps *et al* 2018b).

Fear in animals is often tested through response to novelty (neophobia) or response to humans (Meagher *et al* 2016). For calves, specifically, most existing human approach test measures have been developed and tested under experimental conditions by the use of test arenas, modification of home pens, fixation or handling scenarios, and the animals are tested either individually or in pairs (de Passillé *et al* 1996; Lensink *et al* 2003; Lerust *et al* 2006). Such tests may be less relevant and less applicable for on-farm measurement as they require the farmer to be present and are costly and arguably time consuming. Moreover, it has been shown that frequency and duration of play behaviour may differ for calves when tested in their home pen compared to a test arena (Mintline *et al* 2012), demonstrating that results from test arena testing cannot always be generalised to the home environment. In order to be robust, animal welfare measures must show high inter-observer agreement and test-retest reliability, and clearly be applicable to production-specific conditions, such as group housing, which is now a legal requirement in Europe for calves older than eight weeks (The Council of the European Union 2008). Only a few studies have focused on developing simple and robust measures of group-housed calf behavioural response to human approach for use on-farm (eg Rousing *et al* 2005; Leruste *et al* 2006). Leruste *et al* (2006) focused on larger groups of post-weaned calves, while Rousing *et al* (2005) studied smaller groups including milk-fed calves. Leruste *et al* (2006) studied four different HAR tests and found varying validity and reliability in the results (detailed argumentation can be found in Leruste *et al* 2006). Rousing *et al* (2005) developed a simple and quick on-farm test of individual calf behavioural response to an approaching human,

Table 1 Predictors used to investigate avoidant behaviour (0–5) in dairy calves.

Predictor	Description	Categories	N	Avoidance score						Mean (± SD)
				0	1	2	3	4	5	
Age	Age (days) of study participants at time of testing	Continuous (range 22–288)	548	152	98	84	77	68	69	2.03 (± 1.75)
Sex	Sex of the study participant	Bull	201	45	33	30	28	27	38	2.36 (± 1.82)
		Heifer	347	107	65	54	49	41	31	1.84 (± 1.68)
Test_order	Order in which the study participant was tested	1	111	13	26	19	14	17	22	2.56 (± 1.71)
		2	109	29	21	18	19	10	12	1.96 (± 1.68)
		3	110	34	19	15	15	12	15	1.97 (± 1.80)
		4	108	34	13	21	15	14	11	1.95 (± 1.73)
		≥ 5	110	42	18	11	14	15	9	1.71 (± 1.75)
Test_order.dich	Dichotomised test order with calves tested first and calves tested after	1	111	13	26	19	14	17	22	2.56 (± 1.17)
		≥ 2 (2, 3, 4, 5)	437	139	72	65	63	51	47	1.90 (± 1.74)
Weaning	Weaning status of the study participants at the time of testing	Not weaned	50	25	5	6	7	5	2	1.36 (± 1.63)
		Being weaned	43	11	8	4	4	7	9	2.35 (± 1.95)
		Weaned	412	99	80	67	61	51	54	2.11 (± 1.72)
		(Missing)	43	n/a						

carried out at feeding time in unrestrained, group-housed calves. In contrast to Leruste *et al* (2006), the study by Rousing *et al* (2005) reported high inter-observer agreement, moderate to high test-retest reliability, and an effect of test-person familiarity, as well as an effect of herd. Rousing *et al* (2005) did not, however, evaluate the animal-related factors that may potentially influence the results. Thus, the objective of this paper was to determine the effects of sex, age and the order in which calves within a pen were tested on the avoidance response of group-housed calves to an approaching human in a test set-up as described by Rousing *et al* (2005).

Materials and methods

Study animals

From January 2006 to March 2008, 548 group-housed calves on 110 farms were tested. All calves were evaluated once for their individual behavioural responses to one and the same trained, unfamiliar human approaching them from outside their home pen. One randomly selected calf pen was tested on each farm which, in turn, met the following inclusion criteria: concentrate feeding of calves from a manger and participation in parallel scientific study of calf health (Gulliksen *et al* 2009). In addition, all study participants were members of the National Cow Health Registration System (NCHRS), encompassing 98% of Norwegian dairy producers. All experimental procedures were in accordance with the regulations controlling experiments/procedures on live animals in Norway, and the study complies with the policies relating to animal ethics. Due to the nature of the experiments, permission from the Norwegian Animal Research Authority was not required.

Dairy calf management varies greatly in Norway, and hence also in the study sample. This makes the typical management system difficult to characterise. However, most calves in conventional dairy farming are separated from the dam immediately after birth and placed in single pens. Here, they are usually fed around 8 l of milk per day from a bottle or teat bucket, in addition to having access to concentrates, hay, silage and water. The calves are usually kept in single pens for the first two weeks of life before being moved to group pens of varying size. Weaning off milk usually takes place between the ages of six and eight weeks.

Calves' individual characteristics were recorded at the time of testing, including: breed, age, sex, weaning stage, and weight. Among the calves tested, 500 (91.2%) were Norwegian Red, 21 (3.9%) were Norwegian Red crosses, six (1.1%) were Holstein, six (1.1%) were Colour-sided Troender and Nordland Cattle, five (0.9%) were other breeds, and ten (1.8%) were unknown. In total, there were 347 (63.3%) heifer calves and 201 (36.7%) bull calves. The mean (± SD) age of the calves at testing was 125.2 (± 44.55) days and ranged from 22 to 288 days. The age range for heifers was 22–288 days and for bulls it was 26–269 days. The ages of the calves were distributed in the following groups: 110 (20.1%) were 22–88 days, 314 (57.3%) were 88–155 days, 107 (19.5%) were 156–222 days, and 17 (3.1%) 223–288 days. The weaning status of each calf was recorded as either not weaned, being weaned, or weaned. The calf is considered to be fully

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weaned once it is no longer fed any milk (or milk replacer). Four hundred and twelve (75.1%) of the calves in the study were weaned, while 50 (9.1%) were not weaned, 43 (7.9%) were being weaned, and 43 (7.9%) were missing information. The mean (± SD) age at weaning for the calves was 71 (± 20) days and ranged from 38–161 days.

The composition of the test groups varied between farms (Table 1). Seventy-four (67.3%) of the test groups consisted of bull and heifer calves housed together, while 30 (27.3%) groups were only heifers and six (5.4%) were only bulls. The differences in age within each test group ranged from seven to 262 days (mean 56.5 [± 40.34] days). In an attempt to standardise the set-up, it was decided to test one pen with five calves on each farm. This was not, however, possible on all farms as not all farms had five calves at the time of visit. On two occasions, an additional sixth calf was tested. One hundred and five (95.5%) farms had five individuals tested, two (1.8%) farms had six individuals tested, two (1.8%) farms had four individuals tested, and one (0.9%) farm had three individuals tested.

Figure 1

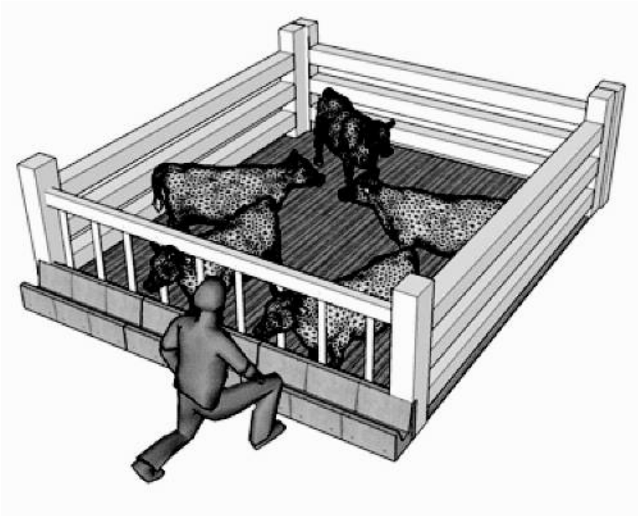

Illustration showing the general set-up of the avoidance test.

Test procedure

Testing of calves was carried out as a slightly modified, three-step procedure as described in Rousing *et al* (2005). Calves were tested in their home pens when fed concentrate in their manger (Figure 1). Although they were tested individually, all calves were present in the pen during the entire test period.

Regular feeding of the calves, including hay and milk-feeding of non-weaned calves, was conducted as normal on the day of testing. However, concentrate, which was normally fed in a between-farm varying and unknown amount to all tested calves on all farms, was withheld from the calves on the test day prior to testing. A few minutes before starting the test, any leftover feed was removed from the manger. At test start, the calves were fed the concentrate normally used on the respective farm in an amount that ensured *ad libitum* access during testing. This meant that the time between last concentrate feeding and amount of concentrate fed at test time varied from farm-to-farm as per each herd, each individual feeding schedule and feeding plan. If a farm had several calf pens, the pen furthest away from the walkways would be selected for testing. This was done to avoid the potential bias of testing only the tamest animals. If the test pen held more than five calves, the test subjects were randomly selected based on ear-tag number before entering the calf barn. At the beginning of the test, the calf manager administered concentrates evenly across the entire length of the manger. The amount provided was large enough to ensure *ad libitum* access to concentrate for all animals during the testing procedure. The brand of concentrate was the same that was normally used on each respective farm. At the time the concentrates were offered, the test person positioned himself approximately 2–3 m from the manger and waited (outside the pen) until all the calves had started eating before approaching each calf in turn. As concentrates had been withheld on the day of testing, the calves tended to approach the manger immediately. The animal furthest to the right of the manger was always tested first, before the tester moved successively to the left. The test person approached each individual calf diagonally from the right in a standardised fashion, ie slowly (around one moderate step per second) and diagonally, facing the calves without making eye contact and keeping arms and hands close to the body. Approximately 1 m from the calf, the test person stopped and remained motionless for 15 s. The test person then reached out, tried to touch the calf's head through the barrier for a few seconds, and then stayed motionless for 15 s. Following this, the test person knelt and remained motionless for 15 s. An attempt was then made, still from outside the pen and through the barrier, to reach out and scratch the calf's head or ears for a few seconds. Avoidance was defined as the head positioned behind the forage fence for more than 15 s or moving to another feed location. The avoidance response of the individual calf was categorised from 0 to 5 (maximal to no avoidance), defined as: (0) Calf avoids the manger after initial approach; (1) Avoidance at 'test person approach start'; (2) Avoidance at 'head touch approach while standing'; (3) Avoidance at 'kneeling down'; (4) Avoidance at 'head touch while kneeling'; and (5) No avoidance at 'head touch while kneeling'. The test was terminated with the first sustained (> 15 s) avoidance behaviour. If the test calf, for example, approached the manger, allowed the test person to approach and touch its head while standing, but withdrew when the test person knelt down, that calf would be given a score of 3. All avoidance responses were scored by the same observer (the test person).

As soon as one calf was given an avoidance score, the procedure was repeated for the next animal at the feeding trough (moving successively from right to left). If the calf about to be tested stopped eating and withdrew from the manger, the test person awaited the resumption of eating (for up to 1 min) before continuing the test. If the calf did not re-approach, that animal was given a score of 0. The set-

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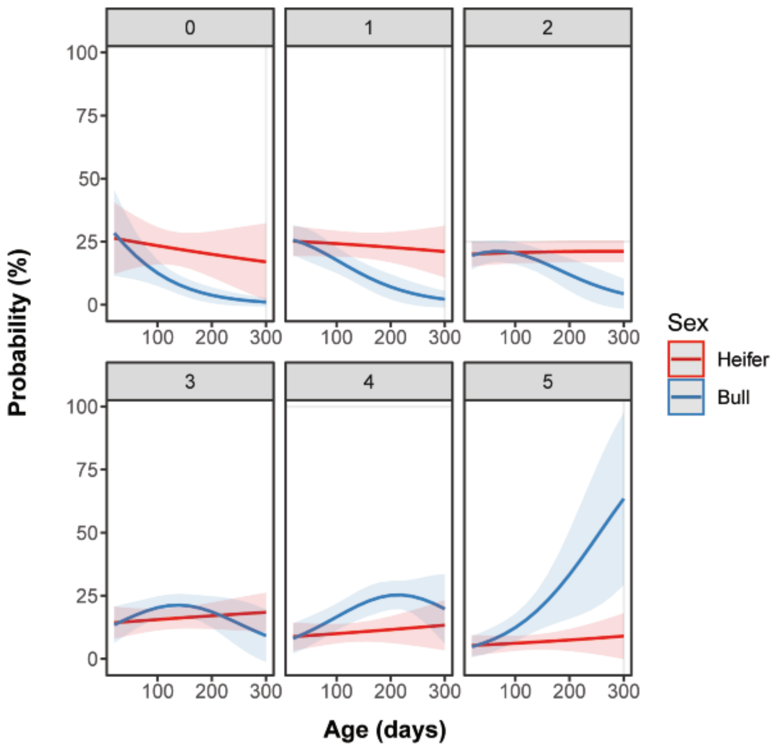
Figure 2



Predicted probabilities with 95% CIs for age and sex for six levels of avoidance response from 0 (most avoidant) to 5 (least avoidant).

up with regards to pen size, fencing, flooring etc, varied among the farms. However, all test pens were sufficiently large to allow the animals to withdraw and all mangers were large enough to allow all animals to eat simultaneously.

Statistical analysis

The influence of the fixed effects (sex, age, weaning stage, and test order) on the categorical avoidance response (scored 0–5) was investigated using mixed ordinal logistic regression models (with the cumulative logit as the link function). Herd (also referred to as pen) was included as a random effect in the model to account for differences between farms. Herd effects were assumed to be independent, identically and normally distributed (IID-normal). For the fixed effects, age was tested as a continuous variable and weaning stage was used as a categorical variable with three categories: not weaned, being weaned, and weaned. Test order was used as a categorical variable with five categories: 1, 2, 3, 4, and ≥ 5. This variable was also used as a dichotomous variable comparing calves tested first and those tested after. Univariable models were used to investigate the effects of each predictor on the avoidance response.

The final model was selected using forward selection beginning with the most significant univariable model, and testing for all possible two-way interactions with the main effects. Weaning status was not included in the multivariable analysis due to missing data from 43 test subjects. Only the dichotomised variable for test order (tested first/not tested first) was used in the multivariable model. The remaining predictors, age, sex and the dichotomous variable for test order were assessed prior to model building for correlations by calculating the *phi* correlation coefficient between two binary variables or the point biserial correlation coefficient between binary and continuous variables. Model fit was assessed using AIC (Akaike's Information Criterion) (Sakamoto *et al* 1986) and performing ANOVA for nested models, whereby main effects were only added if they or their interactions contributed significantly ($\alpha = 0.05$) to the predictive ability of the model. All models were implemented using the 'ordinal' package (Christensen 2015) in R (R Core Team 2016).

Results

The distribution of heifer and bull calves in the six avoidance categories is shown in Figure 2. More than a quarter (27.7%) of all calves were given an avoidance score of 0, indicating that they withdrew from the manger during testing. The number of calves in each category decreased with increasing avoidance scores: 17.9% were scored 1, 15.3% scored 2, 14.1% scored 3, 12.4% scored 4, and 12.6% scored 5.

The results of the univariable analyses of the predictors with herd as a random effect are included in Table 2. The analysis did not detect a significant effect of age on avoidance response. Sex was found to be significantly associated with avoidance response ($P < 0.001$), with heifer calves displaying more avoidant behaviour than bull calves. Test order was also significant, with the calves tested after the first individual showing significantly higher avoidance responses ($P < 0.001$). Finally, weaning was also found to be significant, with individuals that were being weaned ($P = 0.018$) or weaned ($P = 0.003$) displaying less avoidant behaviour.

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Table 2 Results of univariable cumulative link mixed models (CLMMs) for the effects of age, sex, test order, and weaning status on avoidance response in calves, with herd as a random effect.

Model	Predictor		Estimate	Standard error	*Z*-score	*P*-value
Univariable models	Age		0.004	0.003	1.564	0.118
	Sex	Bull	0.818	0.199	4.111	< 0.001
	Test_order	2	–0.735	0.247	–2.947	0.003
		3	–0.800	0.252	–3.177	0.001
		4	–0.855	0.250	–3.422	< 0.001
		5	–1.246	0.258	–4.831	< 0.001
	Test_order.dich	≥ 2	–0.899	0.196	–4.584	< 0.001
	Weaning status	Being weaned	1.142	0.486	2.357	0.018
		Weaned	1.127	0.383	2.946	0.003

Estimates for the threshold coefficients for each model and the variance of the random effect are not shown.

Table 3 Parameter estimates for the final cumulative link mixed model (CLMM) for avoidance response of calves including the estimates for the threshold coefficients, the predictors, and the variance of the random effect of herd.

Model	Threshold value	Estimate	Standard error	*Z*-score	*P*-value
Multivariable model	0/1	–1.522	0.470	–3.241	
	1/2	–0.375	0.464	–0.807	
	2/3	0.536	0.467	1.147	
	3/4	1.450	0.474	3.060	
	4/5	2.547	0.488	5.220	
	Fixed effect	**Estimate**	**Standard error**	***Z*-score**	***P*-value**
	Test_order.dich	–0.963	0.198	–4.866	< 0.001
	Age	0.002	0.003	0.670	0.503
	Sex (Bull)	–0.327	0.574	–0.570	0.568
	Age × Sex (Bull)	0.011	0.005	2.368	0.018
	Random effect	**Variance**	**Standard error**		
	Herd	2.253	1.501		

The final multivariable model (Table 3) included the fixed effects of the dichotomised variable for test order, age, sex, and an interaction term between age and sex.

The results of the multivariable model showed that animals that were not tested first had lower response scores, corresponding to a higher avoidance response, than animals that were tested first (OR = 0.38, 95% CI = 0.09, 0.55; $P < 0.001$). Furthermore, the analysis revealed that younger bull calves had a higher avoidance response than older bull calves, while the avoidance response of heifers was relatively stable regardless of age (Figure 2).

Discussion

It was found that heifer calves, irrespective of age and test order, in general showed more avoidant behaviour towards an unfamiliar approaching human than bull calves, with older bull calves showing the least avoidant behaviour. Windschnurer *et al* (2009) tested five to 20 month old bull calves in a similar set-up as in the present study. They found, in accordance with the current results, that the older bull calves showed less avoidance than the younger bull calves. Based on these findings, Windschnurer *et al* (2009) suggested that the avoidance response might be associated with sexual maturity in the bulls. Puberty hits bull calves at 37–50 weeks of age (8.5 to 11.5 months) (Rawlings *et al* 2008). Even so, testosterone secretion commences between 3.5 and 5.5 months (Lacroix & Pelletier 1979; Bollwein *et al* 2016), and it has been firmly established that testosterone reduces fear in animals (van Honk *et al* 2005; Oyekunle *et al* 2012).

In this study, calves tested later in the group avoided at an earlier stage when the human approached. There are three potential explanations that could account for this phenomenon either alone or in combination. Firstly, there is an effect of food motivation which weighs a calf's avoidance behaviour at human approach with the calf's food motivation. The duration of testing a group of five calves was approximately 5 min. It is possible that motivation to feed declined with increased feed intake during testing. Second is the effect of habitation, whereby a calf's avoidance behaviour at human approach is influenced by their possible curiosity towards the unfamiliar human. The novelty value of the human approaching is obviously declining during re-testing. Effects of habituation in tests of cow and calf behavioural responses have been reported, for example, in Rousing and Waiblinger (2004) and Leruste *et al* (2006). Thirdly, there is a social effect. The pattern of increasing withdrawal during the test could also be an effect of the calves' influence on each other. For example, Boissy *et al* (1998) reported that when heifers are exposed to a novel environment, they show less tendency to feed in the presence of a stressed partner than in the presence of one that is non-stressed. As social facilitation is a 'copycat' phenomenon occurring in many acts of free movement within a group (Albright & Arave 1997) it is possible that the effect of test order found in the present study is a cumulative effect of avoidance in the group. Weaning status was a significant predictor of avoidant behaviour in the univariable stage and should probably be further investigated as it could not be included in the current multivariable model due to missing data. The observed effect of weaning status on avoidant behaviour, may be due to altered motivation to obtain concentrates. As the rumen develops in the young calf, the animal relies more heavily on solid feed for nutrition (Khan *et al* 2016). With increasing bodyweight, feed intake also increases, thereby increasing the motivation to consume concentrates (Schütz *et al* 2018).

A number of factors beyond sex, age and test order may influence the animals' response in a human approach test. For instance, it has been shown that the number of pen-mates has an effect on fearfulness and hence avoidance behaviour (Lensink *et al* 2001). It was not possible to test this effect in the current study however as over 95% of the farms had a group size of five. Other potential confounding factors include calf health status (Cramer & Stanton 2015), previous feeding regime (Jago *et al* 1999), time spent with the dam (Krohn *et al* 1999) and previous experience with people (Veissier *et al* 2000). To account for differences between herds, herd was included as a random effect in the statistical models. In practice, the effect of herd means that animals on some farms are less avoidant than animals on other farms, likely due to the quality of the HAR (Rushen *et al* 1999b; Waiblinger *et al* 2006; Zulkifli 2013). For instance, Ellingsen *et al* (2014) showed that farmers with a positive handling style had calves that were more confident and social, while farmers with a negative handling style had calves that were more tense and frightened. Studies have also shown that handling style affects approach-avoidance in extensively held cattle (Le Neindre *et al* 1996) and group-housed dairy calves (de Passillé *et al* 1996; Schuetz *et al* 2012).

The approach-avoidance behaviour was tested in a scenario of intended feed uptake motivation. Similar test designs have also been applied in other studies, including Waiblinger *et al* (2003) (group-housed dairy cows), Windschnurer *et al* (2009) (group-housed bulls), and Rousing *et al* (2005) (group-housed calves). For practical reasons, standardising the time of testing relative to the last feeding — and to the amount of concentrate normally fed — was not possible in the current study. Since tests were held outside of regular feeding times, and because the amount of concentrate fed may have deviated from the normal amount, calves' satiety levels most likely varied from farm-to-farm. This may have influenced the test results. If a group of calves was tested shortly before their usual meal-time they may have been extra motivated to eat the concentrates and not withdraw from the test person during the test, compared to those animals tested shortly after their regular mealtime. In the opposite scenario, in the case of calves being tested shortly after their usual feeding times, it could also be argued that animals in category 0 (Calf withdraws from the manger before approach) did not resume feeding because they were not hungry, regardless of the level of fearfulness. However, concentrate is generally regarded as highly attractive and providing *ad libitum* access during the test procedure should be a strong motivator for the calves to approach and stay at the manger. Hence, category 0 is still likely to be a result of fearfulness, not differences in hunger.

Animal welfare implications

Dairy calves are routinely handled and moved. For this reason, a good HAR is crucial to achieve positive animal welfare. A poor HAR may result in fearful animals, reduced welfare and, over time, reduced health and production. Fear in animals is often tested through response to novelty (neophobia) or response to humans (Meagher *et al* 2016). Isolating the factors that influence fear in animals is, hence, the first step in reducing fear and increasing animal welfare. As shown in this study, it is necessary to include individual attributes in the model when analysing effects of other changeable management factors on human approach. The study's contribution to animal welfare is aimed at increasing the knowledge of factors influencing human approach tests which are widely used in existing on-farm welfare assessment protocols.

Conclusion

The results of this study suggest that the individual behavioural response of group-housed calves to human approach, whilst feeding on concentrates in their home pen, is affected by the sex and test order. Moreover, the avoidance response of bull calves depended on age, with older bulls showing less avoidant behaviour than younger bulls.

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