

Does the avoidance distance test at the feed barrier have scientific validity for evaluating reactivity to humans in Limousin breeding bulls?

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- 1 Does the avoidance distance test at the feed barrier have scientific validity for evaluating
- 2 reactivity to humans in Limousin breeding bulls?
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- 15 Abstract

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- 16 Testing beef bull reactivity to humans is a key challenge for improving beef cattle reactions to
- handling, but the process can be dangerous and requires skill in cattle handling. Testing
- avoidance distance at the feed barrier (ADF) would be a safer option than test procedures
- involving exposure to free moving animals. Here we tested ADF for test re-test consistency
- 20 one week apart and for convergent validity with three other tests involving humans where
- bulls were free to move. We also tested the relationship between ADF score and growth
- 22 performances. This observational study used 115 Limousin bulls evaluated on-farm around
- 23 weaning (8 months) and at the French national evaluation and qualification station for
- Limousin-breed young bulls, where they were housed from 10 to 15 months of age for a
- 25 period of control. Qualitative on-farm behavioural scores (BeF), on-station behavioural scores
- 26 (BeS) and on-station docility scores (Do) were collected during the routine pedigree bull
- selection process. Three repetitions of the ADF test were performed, in three weeks before the
- end of the period of control. Standardised 120-day and 400-day weights were calculated and
- 29 correlated to behavioural scores. ADF showed moderate consistency through the three

- 30 repetitions (overall intraclass correlation coefficient=0.54). Mixed-effect ordinal logistic
- 31 regressions were performed to evaluate the links between ADF score and other behavioural
- data. ADF score was positively related to other scores collected on-station (ADF–BeS,
- p<0.01; ADF–Do, p<0.05). Animals with lower ADF scores also had heavier predicted 120-
- day and 400-day weights (p<0.01). Our results suggests that ADF shows consistency with
- 35 other tests involving humans and is related to key predicted weight outcomes at genetic
- selection. The ADF test emerges as a promising option for phenotyping individual
- 37 responsiveness to humans.
- 38 Keywords: Avoidance distance; docility test; beef cattle; temperament; human–animal
- 39 relationship

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1. Introduction

- The number of cattle per worker is increasing in many countries, (Gargiulo et al., 2018;
- 42 Veysset et al., 2014), potentially reducing the relational proximity between livestock and
- farmers. The risk, depending on farmers' attitudes towards animals, is that if human–animal
- 44 interactions essentially only occur during handling, then animals will become increasingly
- 45 fearful of humans (Destrez et al., 2018; Hemsworth and Coleman, 2011). For stockpeople,
- 46 handling fearful animals is an occupational health and safety hazard (Ceballos et al., 2018;
- 47 Gutierrez-Gil et al., 2008), but cattle fear to humans could reduce animal welfare and
- productivity (milk yield, growth, feed efficiency, meat quality) (Haskell et al., 2014;
- 49 Hemsworth and Boivin, 2011; Olson et al., 2019).
- The reactivity of cattle to humans results from a dynamic learning process based on prior
- 51 human–animal interactions (Waiblinger et al., 2006). This process interacts with genetic
- 52 traits: animals show inter-individual behavioural differences to human presence and handling
- 53 (calm, docile, distressed, struggling to escape, and so on) that are repeatable over time and
- 54 across situations and partly genetically inherited (see Haskell et al., 2014 for review).

A number of genetic selection programmes use protocols to evaluate cattle reactivity to humans (Haskell et al., 2014; Phocas et al., 2006). These protocols feature various tests of responses to humans and to handling involving direct human presence, but also responses to restraint in handling facilities (Haskell et al., 2014; see Waiblinger et al., 2006 for reviews). For example, in France, young Limousine breeding bulls are first evaluated on their reaction to human approach in their original farm (Vénot et al, 2015). They are then gathered in Lanaud station where a routine-practice "docility test" is performed to select breeding bulls and improve reactivity to humans (Le Neindre et al., 1995, Phocas et al., 2006). The docility test, performed since 1992, involves direct exposure to human presence after a short period of social separation, where an experienced but unfamiliar handler attempts to restrain the bull in a corner of a corral pen. However, this test is time-consuming, stressful, and poses a safety hazard with risk of injury for both the handler and the animal (Sant'Anna and Paranhos da Costa, 2013). Moreover, it requires skills in cattle handling, especially with bulls, and a specific testing area. Safer tests, possibly performed without moving the animals, would be by far a better option. For example, Waiblinger et al. (2003) developed a test called "avoidance distance at the feeding rack" (ADF) for evaluating the human–dairy cattle relationship. This test evaluates the distance to an unknown human approaching from outside the freestall before a cow shows an avoidance reaction (head, leg). It has been transformed in a four-point scale to evaluate the human-animal relationship in the protocol for Welfare Quality® assessment on dairy and fattening cattle. This avoidance distance test has been used for dairy cattle (see Ebinghaus et al., 2017, for review), but more rarely for bulls (see Windschnurer et al., 2009, on fattening bulls). As for dairy cattle, Windschnurer et al. (2009) reported that the test scores can be related at farm level to stockperson attitudes and behaviour towards the animals. For breeding bulls, there is still a lack of key proof of its scientific validity at an individual level as

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described by Waiblinger et al, (2006) (i.e. relationship with other tests where animals are free to move in human presence, and other elements). Here, to address this gap, we evaluated the test-retest consistency of avoidance distance test at the feed barrier (ADF) and its convergent validity with other handling situations routinely performed in the Limousin breed selection process. Relationships between cattle reactivity and productivity have already been demonstrated in other studies, so we also assumed a negative relationship between ADF and weight and growth performances.

2. Animals, materials and methods

This observational study was performed between February and June 2018 at the Lanaud station (Boisseuil, France) for the Limousin beef breed. Every year, this national breed station evaluates about 750 candidate young bulls pre-selected early based on morphological criteria. These candidate animals come from a network of a thousand private farms across all of France and Luxembourg. Data were obtained on a subsample of 115 bulls present during the whole observation period and evaluated via routine practices already performed in the Limousin breed selection process. Our study did not specifically impose stressful situations for the animals, and so, institutional animal care and use committee approval was not required under European regulations.

The bulls were originally born within 83 different farms and were the products of 104 different sires. Bulls entered at station at 303 ± 27 days of age and 445 ± 48 kg body weight. These data were in line with the mean age (300 ± 22 days of age) and body weight (449 ± 43 kg) of the four last year of station controls (personal communication). They were then housed on-station a period of five months in $5m \times 8m$ freestalls in groups of 6 to 8 animals that were never more than six days old apart. Diet was composed of 22% straw, 22% hay, 14% barley, 14% triticale, 6% liquid protein nitrogen feed, and 22% nonprotein nitrogen supplement that included minerals. The average daily gain (ADG) goal was 1300 g a day. Rations were

distributed two times a day, at between 08:30–10:00 and between 16:30–17:30. During feed 106 distributions, bulls were headlocked at the feed barrier for half an hour, and the stall floor was covered with straw. Straw was brought with a tractor, then scattered around by humans who 107 used this time to check whether bulls were uninjured or ill and deliver any care needed. 108 Visitors were regularly present in the barns, but always outside the rearing pens and never 109 approaching close to the bulls. 110 2.3. Testing procedure 111 112 The testing procedure is presented here in a way that reflects the objective of this study, and not the chronology of events. The interested reader can see Figure 1 for a chart setting out the 113 timeline chronology of the testing procedure and Table 1 for a summary of the behavioural 114 tests performed. 2.3.1. Avoidance distance at the feed barrier (ADF) 116 During the fourth month at the station, an avoidance distance test at the feed barrier was 117 performed three times (ADF1, ADF2, ADF3), each at one-week intervals. Bulls were 395 ± 118 27 days old when the first ADF was performed (Fig.1). 119 The test procedure followed the Welfare Quality® assessment protocol for cattle (2009). 120 ADF1, ADF2 and ADF3 were performed at between 09:00–10:00 in the morning, at least 10 minutes after the feed delivery. Animals were headlocked at the feed-rack system (Confort S, Cosnet®). The feed-rack system allowed the bulls to show avoidance and make head 124 movements but not to move away from the human (Fig. 2). A single experimenter wearing the 125 same dark green overalls and rubber boots each time performed all three tests. She was

unfamiliar to the bulls but trained to perform the ADF testing in a standard manner (regular

whether the tested animal looked at her, she approached it at a speed of one step per second,

starting face-on from a distance of 3 m, with one arm at 45° in front of the body and the back

walking manner, distance score evaluation; Welfare Quality, 2009). After waiting to see

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of the hand facing the bull. The experimenter stopped walking as soon as the bull showed avoidance or let itself be touched on the nose/muzzle. Avoidance was defined as stepping back or turning the head more than 45°. Avoidance distance was defined as the distance between the experimenter's hand and the bull's muzzle. The bulls were scored on the one-to-four scale as defined in the Welfare Quality® assessment protocol for cattle (Welfare Quality, 2009). Bulls that were touched were scored 1, bulls that let the experimenter approach to within under 0.50 m were scored 2, bulls that let the experimenter approach to between 0.50 m and 1 m were scored 3, and bulls that did not let the experimenter approach any closer than 1 m were scored 4. One in every two animals was tested first, and then the remaining ones were tested. The animal was retested later if its reaction was unclear or if its neighbours showed avoidance before the tested animal reacted.

2.3.2. Evaluation of behaviours towards a human being during the on-farm morphological
 assessment (BeF)

At 227 \pm 32 days old (Fig. 1), within the farms the young bulls on their 'home' farms were scored under the classic national body scoring evaluation process (Idele and FGE, 2014). An unknown trained technician visually assessed and recorded their conformation, size and health of limbs, as well as their behaviour (BeF) while he/she moved around the animal. Other key parameters were collected, i.e. whether the animal was weaned, whether the test was conducted on-pasture or in-freestall, and whether the bull's dam was present. Assessments were performed within the group of bulls, and 39 purpose-trained technicians collected behavioural measures. The different behaviours and their associated scores are described in Vénot et al. (2015) and reported in Table 2.

2.3.3. Evaluation of the behaviours towards a human being during the on-station morphological assessment (BeS)

Four months after admission to the station (at 424 ± 27 days old; Fig.1), the bulls were evaluated for morphology and for behaviour (BeS) following the same process as for BeF. During this second evaluation, one unfamiliar technician individually led the animal for tested to a $10m \times 2.5m$ pen built within the freestall. A partially-opened metal fence separated this pen from the freestall where the animal's in-group peers remained visible. The test was performed without coercion on the animals, i.e. bulls were free to move while technicians performed the body scoring assessment. Three trained technicians observed each animal's behaviour and gave it a consensus score based on the most common behaviour shown according to Table 2. BeF and BeS have been routinely performed since 2011 (Vénot et al., 2015).

2.3.4. Docility test

The station has performed a docility test (Do) as part of routine practice since 1992. The current test, which was adapted from the one developed by Le Neindre et al (1995), is used to eliminate the most dangerous animals. Here the docility test was conducted three weeks after the bulls arrived at the evaluation station (at 323 ± 27 days old; Fig.1). For each test, the bull was separated from its peers and led into a 4m × 4m pen. Two solid panels formed one corner of this pen whereas the rest of the pen was made with partially-open panels. For the test, the animal was left alone in the pen for the first 10 seconds, then the technician entered and stood motionless in the centre of the pen. After 30 seconds stood motionless, the technician tried to contain the bulls for 3 seconds in the 2m × 2m corner of the pen that was formed by the solid panels opposite the peers' pen. The technician had 60 seconds to try to corner the bull, and then went to the opposite corner and stood still for 30 seconds. After these 30 seconds, the technician re-attempted to contain the bull in the corner. The test was then over. The bull was given two scores corresponding to the two handling phases. The scores range from 1 to 4, with half-points possible in cases of intermediate

reaction. The bull was scored 1 if the technician contained it in the corner, 2 if the bull never stopped slowly shuffling around, 3 if the bull never stopped quickly shuffling around, and 4 if the bull charged the technician or attempted to escape by jumping over the pen fencing. The final score is the average of the two stages. The test was performed alternately by three trained technicians unfamiliar to the bulls but experienced in handling beef cattle. The technicians performed this test for all bulls entering the evaluation station. One technician tested 6 to 8 animals before switching for another technician to take over. Tests were stopped if there was a clear risk of injury to a technician or the bull if a bull attacked or tried to violently escape from the testing area. Seven animals that scored '4' were eliminated from the controls at this step and returned to their farms, and therefore were ruled out of inclusion in the analysed dataset.

2.4. *Growth performance*

Animals were weighed at the beginning (at age 331±27 days) and at the end (at age 421±27 days) of the evaluation period. Average daily gain (ADG) over this period was calculated. Behavioural data was cross-compared against the key weight values for genetic selection rather than using actual weights (France Génétique Elevage, 2009). The key weights i.e. the 120-day weight and 400-day weight were calculated using the following formula.

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$$Weight = \left(\frac{(A - A2)(W2 - W1)}{A2 - A1} + W2\right)$$

where *A* is the reference age in days (120 or 400), *AI* is real age at the first weighing,

199 *A2* is real age at the second weighing, and *W* is weight at the first (*WI*) and the second

200 weighing (*W2*).

2.5. Statistical analyses

Data were analysed using R software version 3.5.0 (R Core Team, 2018).

Wilcoxon–Mann–Whitney or Kruskal-Wallis tests were used to study the influence of dam's presence (absence/presence), housing conditions (pasture/indoor) or weaning status on BeF and the technician identity effect on docility score. The farm or technician effects were not tested for BeF because the number of bulls evaluated per farm or by each technician was too low.

ADF test-retest consistency was assessed by calculating the intraclass correlation coefficient

(ICC) of ordinal logistic regressions with random effects. The fixed effect was the test number (one to three) and the random effect was the animal identifier. On-station freestalled bull groups and age were also tested but showed no significant effects on ADF and were not considered in the final model. The model was run using the 'ordinal' package (Christensen, 2019), and ICC was calculated with the 'performance' package (Lüdecke et al., 2020).

Relationships between ADF and routinely collected behavioural data were evaluated using ordinal logistic regressions with random effects run using the ordinal package. The fixed effect was test number and behavioural test (BeF, Do or BeS) and the random effect was animal identifier. We checked for normality of the residuals using a quantile–quantile plot, and we checked the homogeneity of the variance graphically (residuals vs. fitted values plot).

Multiple linear regressions were performed to evaluate the relationships between performance data and mean ADF score (mADF). Age at the first ADF test was added to these regressions as a fixed effect. On-station freestalled bull groups were also tested as a random effect but had no significant effects and were not considered in the final model. Normality and homogeneity of the variance were checked graphically.

3. Results

3.1. Description of the dataset from the behavioural tests

- Figures 3 to 5 give the distribution for each recorded variable. All variables covered nearly
- 228 the whole range of the score scales. Median ADF score was 2 (Fig. 3).
- The behavioural scores (BeS, BeF) had similar distributions between the on-farm and on-
- station performance tests (Fig. 4), with immobility being the most common behaviour (Be=2).
- BeF ranged from 1 (i.e. slowly approaching) to 4 (i.e. walking away fast). BeS ranged from 1
- to 6 (i.e. a state of heightened alertness), but very few animals were scored over 4. BeF scores
- were unaffected by weaning status ($W_{23,92} = 1192.5$, p=0.30), place of test (pasture vs freestall,
- $W_{76,39}=1395.5$, p=0.57) and presence vs absence of the bull's dam ($W_{35,80}=1496.5$, p=0.62).
- In the docility test, about 60% of bulls were rated 2 or less, which corresponds to animals that
- either let themselves be cornered in the pen or at least moved slowly during the first attempt
- 237 (Fig. 5). Observed animal reactions covered the full scale: about 30% of bulls let themselves
- be cornered (scores 1 and 1.25) while 20% systematically showed fearful reactions during the
- test. Technician identity had no influence on docility score (K=2.204, p=0.33).
- 240 3.2. Consistency of ADF
- ADF scores were unaffected by age or freestall groups (P>0.10). ADF decreased significantly
- through the repetitions (table 3) but the three repeated measures were significantly related
- 243 (P<0.001, table 3) and the overall ICC was 0.54. Therefore, we used the mean of the three
- repetitions (mADF) in order to test its relationship with the performance data.
- 245 *3.3. Consistency between ADF and other behavioural tests*
- Table 3 reports the results of the mixed-effect ordinal logistic regressions between each
- routinely-collected behavioural data and ADF. ADF score was positively related to docility
- 248 score (p=0.018) and to BeS (p=0.0060) but not to BeF (p=0.99).
- 3.4. Relationship between ADF and weight performances

Table 4 reports the results of regressions between performances and mADF. mADF was slightly but significantly negatively linked to 120-day weight and 400-day weight (p<0.01), i.e. heavier animals have lower ADF scores. There was no significant relationship between ADG and ADF.

Discussion

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Our study shows that the ADF test is discriminant among young Limousin bulls at the testing station and is moderately consistent over at least a three-week period. ADF test data also shows degrees of consistency with other data routinely collected on-station on behavioural reactions that involve human interaction with free-to-move animals. ADF test data also appears slightly but positively related to indicators of higher growth performances classically used in the genetic selection process. Consistency and scientific validity are two important aspects to consider when developing a test to evaluate animal reactions to humans (Waiblinger et al., 2006). The levels of consistency are moderate in our study but similar to those observed in other studies in dairy cattle or fattening bulls (Ebinghaus et al., 2017; Windschnurer et al., 2009). This result suggests that the ADF can be fairly confidently used to characterise bulls' responses when approached by a human in a standardised manner. Individual response to the test could have been socially influenced by the neighbouring bulls (Munksgaard et al., 2001). However, as prescribed in the Welfare Quality® assessment protocol (2009), we tested every two animals in order to limit potential social influences, as test was performed in their home pens. In addition, we did not observe a freestall-group effect in our statistical models, which further confirms that we effectively evaluated individual reactivity to human approach. Our results highlight that animals with lower ADF scores were also easier to handle during

tests performed individually on-station (docility test and morphological assessment (BeS)).

The number of studies investigating the relations between several tests involving direct human presence in different contexts remain very limited. Most relevant studies in beef cattle have compared different handling situations (exit velocity score, animal reaction to restraint in a crush, etc.; see Haskell et al., 2014, for review) without clearly controlling human proximity. The relationships between avoidance distance and docility test in our study confirmed a preliminary study conducted in the same conditions with bulls just arriving at the station (Windschnurer et al., 2008b). Our findings are also in line with Windschnurer et al. (2008a) and Ebinghaus et al. (2017) who observed moderate-to-high correlations between ADF test scores and other tests involving tactile contact with free dairy cattle. This study therefore brings argument for scientific convergent validity of the ADF for evaluating beef bull response to humans. Convergent validity implies convergence across independent measures that are conceptually related, in this case reactivity to humans (Waiblinger et al, 2006). The conceptual convergence between the tests performed in our study is based on the concept of flight distance, defined by Grandin (2015) as an individual surrounding area within which intrusion provokes a flight reaction. The BeS test involved technicians turning the bull around to observe it, and the docility test involved a technician attempting to approach and restrain the bull in a corner of the pen. The calmest animals during all these tests can be considered as animals that will accept human proximity in all other situations. It is also instructive to note that significant relations between tests were observed not only between ADF and BeS performed within one month before the end of the testing process but also with the docility test performed three months earlier. This result suggests consistency in bull responses to humans over the on-station bull-testing period, in line with Curley et al. (2006) who demonstrated test–retest (120-days apart) consistency in beef cattle reactivity in a handling facility.

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Behavioural responses to humans were also collected earlier during morphological assessment at the animals' farms of origin (BeF). However, our results did not suggest a significant relationship between ADF and BeF. This could result from the diversity in environmental testing contexts (83 farms providing 115 animals) or among technicians (n=39), and thus a lack of standardisation in the environment or among technician-led processes despite regular training. However, we did find no significant influence of a number of potential environmental effects (Waiblinger et al., 2006 for review), such as weaning status (yes or no), presence of the dam near the calf during testing, or housing conditions (pasture vs indoors). This could also simply be due to the delay between the on-farm BeF tests and the following tests performed much later on-station. Whatever the reasons, the BeF performed at early age did not appear predictive of on-station ADF scores. This study found slight but significant relationships between ADF test results and 120-day and 400-day liveweights. These weights at precise ages indicate the growth potential of the animal, which makes them valuable for evaluating genetic potential (Bishop, 1992; Pabst et al., 1977). To our knowledge, this is the first time these parameters have been related to animal responsiveness to humans. Our results linking weights to avoidance distance concur with another study linking the flight speed test to growth performance in 1,350 purebreed and crossbreed Nellore cattle (Braga et al., 2018). This favourable relationship in term of performances could be explained by the fact that the most reactive animals lose energy by reacting more frequently to environmental stimuli, to the detriment of their growth (Llonch et al., 2016). Fearfulness of humans may also affect animals in several situations, for example, when human presence reduces animal ability to eat sufficiently (Haskell et al, 2014). As relationship with ADF was found only with key weights but not with ADG, we hypothesised that early factors before the admission of animals in station, such as genetic or initial farming

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conditions (e.g., indoor or free-range system) had consequences on growth and reactivity to human (see Haskell et al, 2014 for review).

Limitations of this study

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This observational study is based on correlations, with about one hundred animals coming from a large number of farms and large number of sires. On the basis of age and body weight at animals' entry in station these last four years, our sampling appears reasonably representative of Limousin bulls tested at the Lanaud station. The Lanaud station is purposedesigned for evaluating bulls in standardised conditions. The farms that provide the bulls to the station differ in herd size, housing, and human proximity from many other countries around the world. In addition, some bulls had to be eliminated (essentially due to overaggressivity in the docility test) before the whole on-station dataset was compiled. The ADF test would be particularly interesting if it could also discriminate the most dangerous animals. A preliminary study found evidence that the ADF test performed on arrival at the station could discriminate aggressive animals (Windschnurer et al., 2008b), but this needs to be confirmed. Repeating samplings over several years, testing bulls (including non-selected bulls) at the feed barrier, possibly on-farm or before performing the on-station docility test would be very useful and could also allow us to better explore environmental factors that influence beef bull reactions toward humans (Waiblinger et al., 2006). It could be also interesting to explore the variability among technicians that regularly test calves on-farm in order to confirm (or disconfirm) the absence of relationship between avoidance and BeF, and maybe also to further improve their training.

Finally, studies have shown heritabilities for the docility test and for behaviours collected

during performance tests (Le Neindre et al., 1995; Vénot et al., 2015). A recent study found

significant a heritability ($h^2 = 0.27 \pm 0.06$) for avoidance distance in dairy cattle (Santos, 2017) but to our knowledge no heritability has been calculated for the ADF test in beef cattle. A large-scale study is now needed to check the feasibility of fitting the ADF test to needs of real-world genetic selection that involves rapidly testing thousands of animals (Haskell et al., 2014). Without moving the animals, and with the presence of a head gate for feeding, the ADF can be done quickly and is safer for use with bulls that can sometimes prove highly reactive during handling. This study highlights the potential value of the ADF test to quickly and safely phenotype breeding bull reactivity to humans, but this can only be confirmed by testing a larger population.

Conclusion

This observational study finds that the avoidance distance test at the feed barrier shows testretest consistency and some scientific elements of validity for evaluating the individual
reactivity of Limousin breeding bulls to humans, and may even also be predictive of
individual growth. However, many questions remain to be resolved before the test can be
proposed for bull selection as a tool to usefully replace other tests that are less safe for
stockpeople and for the animals.

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Declarations of interest

372 None

373	References
374	Bishop, S.C., 1992. Phenotypic and genetic variation in body weight, food intake and energy
375	utilisation in Hereford cattle II. Effects of age and length of performance test. Livest.
376	Prod. Sci. 30, 19–31. https://doi.org/10.1016/S0301-6226(05)80018-1
377	Boivin, X., Marcantognini, L., Trillat, G., Godet, J., Brule, A., Boulesteix, P., Veissier, I., de
378	Theix, I., 2006. Docilité des veaux limousins et représentations de cette docilité chez
379	les éleveurs/sélectionneurs. Docility of limousine calves and breeders' attitudes
380	towards this docility. Presented at the Rencontres Recherches Ruminants, p. 1.
381	Braga, J.S., Faucitano, L., Macitelli, F., Sant'Anna, A.C., Méthot, S., Paranhos da Costa,
382	M.J.R., 2018. Temperament effects on performance and adaptability of Nellore young
383	bulls to the feedlot environment. Livest. Sci. 216, 88-93.
384	https://doi.org/10.1016/j.livsci.2018.07.009
385	Ceballos, M.C., Sant'Anna, A.C., Boivin, X., Costa, F. de O., Carvalhal, M.V. de L.,
386	Paranhos da Costa, M.J.R., 2018. Impact of good practices of handling training on
387	beef cattle welfare and stockpeople attitudes and behaviors. Livest. Sci. 216, 24–31.
388	https://doi.org/10.1016/j.livsci.2018.06.019
389	Christensen, R.H.B., 2019. ordinal—Regression Models for Ordinal Data. R package version
390	2019.12-10. https://CRAN.R-project.org/package=ordinal.
391	Curley, K.O., Paschal, J.C., Welsh, T.H., Randel, R.D., 2006. Technical note: Exit velocity as
392	a measure of cattle temperament is repeatable and associated with serum concentration
393	of cortisol in Brahman bulls1. J. Anim. Sci. 84, 3100–3103.
394	https://doi.org/10.2527/jas.2006-055

395	Destrez, A., Haslin, E., Boivin, X., 2018. What stockperson behavior during weighing reveals
396	about the relationship between humans and suckling beef cattle: A preliminary study.
397	Appl. Anim. Behav. Sci. 209, 8-13. https://doi.org/10.1016/j.applanim.2018.10.001
398	Ebinghaus, A., Ivemeyer, S., Lauks, V., Santos, L., Brügemann, K., König, S., Knierim, U.,
399	2017. How to measure dairy cows' responsiveness towards humans in breeding and
400	welfare assessment? A comparison of selected behavioural measures and existing
401	breeding traits. Appl. Anim. Behav. Sci. 196, 22–29.
402	https://doi.org/10.1016/j.applanim.2017.07.006
403	France Génétique Elevage, 2009. Règlement technique du Contrôle Officiel des Performances
404	des Bovins allaitants en ferme. (No. 4.0). France Génétique Elevage, Paris.
405	Gargiulo, J.I., Eastwood, C.R., Garcia, S.C., Lyons, N.A., 2018. Dairy farmers with larger
406	herd sizes adopt more precision dairy technologies. J. Dairy Sci. 101, 5466–5473.
407	https://doi.org/10.3168/jds.2017-13324
408	Grandin, T., 2015. How to improve livestock handling and reduce stress, in: Improving
409	Animal Welfare: A Practical Approach. CAB International, Cambridge, UK, pp. 69-
410	95.
411	Gutierrez-Gil, B., Ball, N., Burton, D., Haskell, M., Williams, J.L., Wiener, P., 2008.
412	Identification of quantitative trait loci affecting cattle temperament. J. Hered. 99, 629-
413	638. https://doi.org/10.1093/jhered/esn060
414	Haskell, M.J., Simm, G., Turner, S.P., 2014. Genetic selection for temperament traits in dairy
415	and beef cattle. Front. Genet. 5. https://doi.org/10.3389/fgene.2014.00368
416	Hemsworth, P., H., Boivin, X., 2011. Human contact, in: Appleby, M.C., Hughes, B.O.,
417	Mench, J.A., Olsson, I.A.S. (Eds.), Animal Welfare, 2nd Edition. CAB International,
418	Cambridge, UK, pp. 246–262.

419	Hemsworth, P.H., Coleman, G.J., 2011. Human-livestock interactions: the stockperson and
420	the productivity and welfare of intensively farmed animals, 2nd ed. CAB
421	International, Wallingford, UK; Cambridge, MA.
422	Idele, FGE, 2014. Guide pratique du pointage des bovins de race à viande, du sevrage à l'âge
423	adulte (No. 0014201001), Résultats. Institut de L'élevage, ISSN: 1773-4738.
424	Le Neindre, P., Trillat, G., Sapa, J., Ménissier, F., Bonnet, J.N., Chupin, J.M., 1995.
425	Individual differences in docility in Limousin cattle. J. Anim. Sci. 73, 2249–2253.
426	https://doi.org/10.2527/1995.7382249x
427	Llonch, P., Somarriba, M., Duthie, CA., Haskell, M.J., Rooke, J.A., Troy, S., Roehe, R.,
428	Turner, S.P., 2016. Association of temperament and acute stress responsiveness with
429	productivity, feed efficiency, and methane emissions in beef cattle: an observational
430	study. Front. Vet. Sci. 3. https://doi.org/10.3389/fvets.2016.00043
431	Lüdecke, D., Makowski, D., Waggoner, P., Patil, I., 2020. performance: Assessment of
432	Regression Models Performance. CRAN. https://doi.org/10.5281/zenodo.3952174
433	Munksgaard, L., DePassillé, A.M., Rushen, J., Herskin, M.S., Kristensen, A.M., 2001. Dairy
434	cows' fear of people: social learning, milk yield and behaviour at milking. Appl.
435	Anim. Behav. Sci. 73, 15–26. https://doi.org/10.1016/S0168-1591(01)00119-8
436	Olson, C.A., Carstens, G.E., Herring, A.D., Hale, D.S., Kayser, W.C., Miller, R.K., 2019.
437	Effects of temperament at feedlot arrival and breed type on growth efficiency, feeding
438	behavior, and carcass value in finishing heifers. J. Anim. Sci. 97, 1828–1839.
439	https://doi.org/10.1093/jas/skz029
440	Pabst, W., Kilkenny, J.B., Langholz, H.J., 1977. Genetic and environmental factors
441	influencing calf performance in pedigree beef cattle in Britain. 2. The relationship
442	between birth, 200-day and 400-day weights and the heritability of weight for age.
443	Anim. Sci. 24, 41–48. https://doi.org/10.1017/S0003356100039180

Phocas, F., Boivin, X., Sapa, J., Trillat, G., Boissy, A., Le Neindre, P., 2006. Genetic 444 445 correlations between temperament and breeding traits in Limousin heifers. Anim. Sci. 82, 805–811. https://doi.org/10.1017/ASC200696 446 R Core Team, 2018. R: A language and environment for statistical computing. R Foundation 447 for Statistical Computing, Vienna, Austria. 448 Sant'Anna, A.C., Paranhos da Costa, M.J.R., 2013. Validity and feasibility of qualitative 449 450 behavior assessment for the evaluation of Nellore cattle temperament. Livest. Sci. 157, 254–262. https://doi.org/10.1016/j.livsci.2013.08.004 451 Santos, L.V., 2017. Quantitative genetic analyses for dairy cow behavior traits and traits 452 453 reflecting human-animal-technic interactions. Justus-Liebig-Universität Giessen, 454 Giessen. Vénot, E., Guerrier, J., Lajudie, P., Dufour, V., Leudet, O., Boivin, X., Sapa, J., Phocas, F., 455 456 2015. Implementation of a French national genetic evaluation of beef cattle temperament from field data. Presented at the Rencontres Recherches Ruminants, pp. 457 107–110. 458 Veysset, P., Benoit, M., Laignel, G., Bébin, D., Roulenc, M., Lherm, M., 2014. Analysis and 459 determinants of the performances evolution of sheep for meat and suckler cattle farms 460 461 in less favored areas from 1990 to 2012. INRA Prod. Anim. 27, 49-64. Waiblinger, S., Boivin, X., Pedersen, V., Tosi, M.-V., Janczak, A.M., Visser, E.K., Jones, 462 R.B., 2006. Assessing the human–animal relationship in farmed species: A critical 463 464 review. Appl. Anim. Behav. Sci. 101, 185-242. https://doi.org/10.1016/j.applanim.2006.02.001 465 Waiblinger, S., Menke, C., Fölsch, D.W., 2003. Influences on the avoidance and approach 466 behaviour of dairy cows towards humans on 35 farms. Appl. Anim. Behav. Sci. 84, 467 23–39. https://doi.org/10.1016/S0168-1591(03)00148-5 468

469	Welfare Quality, 2009. Welfare Quality - Assessment protocol for cattle. Welfare Quality
470	Consortium, Lelystad, Netherlands.
471	Windschnurer, I., Boivin, X., Waiblinger, S., 2009. Reliability of an avoidance distance test
472	for the assessment of animals' responsiveness to humans and a preliminary
473	investigation of its association with farmers' attitudes on bull fattening farms. Appl.
474	Anim. Behav. Sci. 117, 117-127. https://doi.org/10.1016/j.applanim.2008.12.013
475	Windschnurer, I., Schmied, C., Boivin, X., Waiblinger, S., 2008a. Reliability and inter-test
476	relationship of tests for on-farm assessment of dairy cows' relationship to humans.
477	Appl. Anim. Behav. Sci. 114, 37–53. https://doi.org/10.1016/j.applanim.2008.01.017
478	Windschnurer, I., Waiblinger, S., Boulesteix, P., Boivin, X., 2008b. Are responses of beef
479	cattle to a human approaching the feed-barrier related to ease of handling? Presented
480	at the 42nd Congress of the International Society of Applied Ethology (ISAE),
481	Wageningen Academic Publishers, Dublin, Ireland.
482	
483	
484	

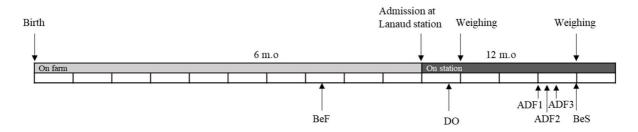


Figure 1. Chronology of the behavioural measures and weighing on animals in farm and during their presence on-station. Each square represents a month. BeF is the behaviour test at farm morphological assessment, DO is the docility test, ADF1, ADF2 and ADF3 are the three avoidance distance tests at the feed barrier, and BeS is the behaviour test at on-station morphological assessment. BeF, DO and BeS are collected routinely and ADF were added for this study.



Figure 2. Young limousin bulls at the feed-rack system ("Confort S", Cosnet®).

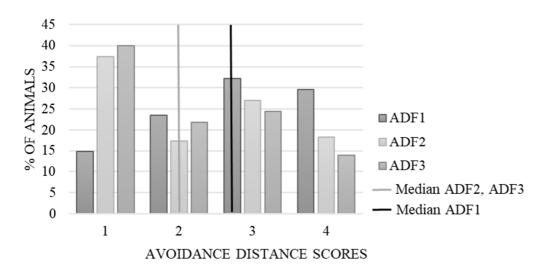


Figure 3. Distribution of the avoidance distance at the feed barrier (ADF) scores for the three repetitions (ADF1, ADF2 and ADF3).

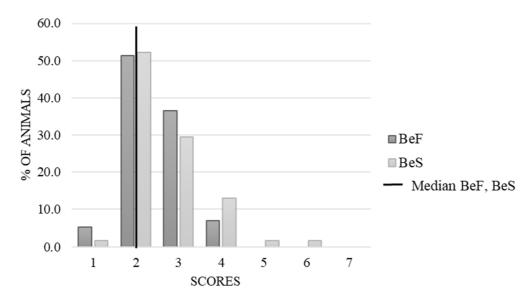


Figure 4. Distribution of the behavioural scores collected during morphological assessment on farm (BeF) and on-station (BeS)

20.0 18.0 16.0 14.0 % OF ANIMALS 12.0 10.0 – Median 8.0 6.0 4.0 2.0 0.0 1.25 2 2.5 2.75 1.5 1.75 2.25 3 3.25 DOCILITY SCORES

Figure 5. Distribution of the docility scores

Table 1. Summary of the behavioural tests performed routinely during the selection process of the Limousin bulls (BeF, BeS and DO) or added in this study (ADF)

Full name of the test	Initial of the test	Location of the test	Age at the test (days)	References
Behaviours towards a human being during	BeF	On farm	227 ± 32	Vénot et al, 2015
the morphological assessment	BeS	On station	424 ± 27	
Docility test	DO	On station	323 ± 27	Adapted from Le Neindre et al, 1995; Boivin et al, 2006.
Avoidance distance at the feed-barrier	ADF	On station	395, 402 and 409 ± 27	Welfare Quality, 2009

Table 2. Scoring scale for on-farm behaviour assessment (BeF).

Score	Associated behaviour
1	Slowly approaches the technician
2	Motionless, indifferent to the experimenter
3	Walks away
4	Walks away fast
5	Runs
6	State of heightened alertness (head movements, gaze fixed on the experimenter)
7	Charges

Table 3. Results of mixed-effect ordinal logistic regressions l between avoidance distance at the feed barrier and other behavioural scores. Example of the R formula for BeF: $clmm(ADF \sim BeF + TestNumber + (1|animal))$

N=115			Estimate	Threshold coefficients	P
ADF~BeF	BeF		0.00 ± 0.13	$0 1 - 1.33 \pm 0.34$	0.993
				$1 2 - 0.49 \pm 0.33$	
				$2 3\ 0.68 \pm 0.33$	
	TestNumber tes	st 1	reference	·	
	te:	st 2	-0.74 ± 0.16		<0.001 ***
	te:	<mark>st 3</mark>	-0.94 ± 0.16		<0.001 ***
	Animal		1.17 ± 1.08		
ADF~BeS	BeS		0.42 ± 0.15	$0 1 - 0.64 \pm 0.29$	0.006 **
				$1 2\ 0.20 \pm 0.29$	
				$2 3 \ 1.37 \pm 0.30$	
	TestNumber tes	<mark>st 1</mark>	reference		
	te:	<mark>st 2</mark>	-0.74 ± 0.16		<0.001 ***
	te:	st 3	-0.94 ± 0.16		<0.001 ***
	Animal		1.05 ± 1.02		
ADF~Do	Do		0.40 ± 0.17	$0 1 - 0.53 \pm 0.37$	0.018 *
				$1 2\ 0.31 \pm 0.36$	
				$2 3\ 1.47 \pm 0.37$	
	TestNumber tes	<mark>st 1</mark>	reference		
	te:	<mark>st 2</mark>	-0.74 ± 0.16		<0.001 ***
	te:	<mark>st 3</mark>	-0.94 ± 0.16		<0.001 ***
	Animal		1.07 ± 1.04		

Table 4. Relationships between growth performances and avoidance distance at the feed barrier scores. Linear model of growth performances \sim mADF + age, where mADF is the mean of the three ADF repetitions.

	\mathbb{R}^2	Model		Estimate	T value	P value
		parameters				
ADG	0.044	$F_{2,112} = 3.61$	Intercept	746 ± 267	2.80	0.006 **
		(P=0.030)	mADF	11.1 ± 20.3	0.55	0.58
			Age (days)	1.8 ± 0.7	2.62	0.010 *
120-day	0.071	$F_{2,112} = 5.34$	Intercept	268 ± 29.2	9.19	< 0.001 ***
liveweight (kg)		(P=0.006)	mADF	-5.9 ± 2.2	-2.66	0.010 **
			Age (days)	-0.1 ± 0.1	-1.85	0.068
400-day	0.087	$F_{2,112} = 6.42$	Intercept	676 ± 51.1	13.26	< 0.001 ***
liveweight (kg)		(P=0.002)	mADF	-12.4 ± 3.9	-3.18	0.002 **
			Age (days)	-0.2 ± 0.1	-1.58	0.12