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On farm welfare assessment of beef cattle using an environmentally-based welfare index and investigation of the human-animal relationship

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1.0 Overall summary

Study 1. Animal welfare index (AWI): an on-farm survey of beef suckler farms in Ireland

Summary

The objectives were to (i) examine the welfare status of Irish beef suckler herds using an animal welfare index (AWI) adapted from a previously validated welfare assessment method (TGI); (ii) determine the influence of the stockpersons' status (full: FT or part-time: PT), their interest in farming and herd size on the AWI; and (iii) compare the AWI with the TGI. Beef suckler farms (196 throughout 13 counties) were assessed once with housed cattle and once with cattle at grass using the AWI**.** Twenty-three of the 196 farms were revisited a year after using the AWI and the TGI. Thirty-three indicators were collected in five categories: locomotion (5 indicators); social interactions (7), flooring (5), environment (7) and stockpersonship (9). Three indicators relating to the size of the farm were also collected. The mean AWI was 65% and ranged from 54% to 83%. The grass period represented 16.5% of mean total points of the AWI. Seventy percent of the farms were rated as "Very Good" or "Excellent". There was no difference ($P > 0.05$) in AWI between FT and PT farmers. PT farmers had greater ($P = 0.01$) "social interactions": calving ($P = 0.03$) and weaning ($P < 0.001$) scores. FT farmers had cleaner animals ($P = 0.03$) and less lameness ($P = 0.01$). The number of animals and the interest of the stockperson were negatively and positively correlated ($P = 0.001$), respectively, with the AWI. A hierarchical classification was performed to examine how the indicators influenced the AWI. Farms could be categorized into three classes, the most discriminating factors for the classes were the interest of the farmer (higher scores when the farmer was more interested in farming) and the number of animals (higher scores when the herds were smaller).

Study 2. Investigation and specificity of behavioural fear responses of heifers to different fear-eliciting situations involving humans.

Summary

This study investigated the specificity of fear responses in housed beef heifers' over time using four behavioural tests; flight, docility, fear and chute tests. The flight, (time to join peers and avoidance distance), docility (isolation and handling) and fear (4 phases; responses of isolated heifers in (i), the absence (ii), the presence, of food and responses to a stationary human (iii) without and (iv) with visual contact of their peers) tests were carried out over three consecutive days, in that order, commencing on day 30 and again on day 80 post-housing. The chute test (movement through a race and agitation of heifers during blood sampling) was performed on day 84 post-housing. Scores (higher scores meant less fearful animals) were assigned to the fear responses. Heifers had the lowest ($P < 0.05$) scores during phases (i) and (iii) of the fear test and the highest ($P< 0.05$) during phase (iv). The most docile heifers during the docility test were the most agitated during the chute test ($P < 0.001$). The fear scores were sTable over time for the docility test but decreased for the fear test. The fear scores when restrained (chute test) were not correlated with other scores except for the agitation. A PCA showed that two components (avoidance of stimulus and general agitation explained 49% of the total variation. In conclusion, this study showed that fear responses of heifers can vary over time and that fear is not unitary but multidimensional. Consequently, fear responses are condition specific and tests assessing fear should consider their specificity.

2.0 Animal welfare index (AWI): an on-farm survey of beef suckler farms in Ireland

2.1 Introduction

Improving animal welfare is an increasingly important aspect of livestock production systems due, to a large extent, increased consumer concern about the source of animal products (www.welfarequality.net; Animal Welfare Eurobarometer, 2005). Indicators for the assessment of farm animal housing were proposed by several researchers and minimal requirements for animal welfare were implemented in the legislation of most European Union member states (EU directives) as reviewed by von Borell (1998), but there is no specific directive on the minimal housing requirements for adult cattle. However codes of practices for the welfare of farm animals are available (EU code of recommendation; Australian Animal Welfare Standards and Guidelines). In order to assess animal welfare on farms in various production systems, different assessment methods have been developed in Europe (Sorensen *et al* 2001).

These methods have taken into consideration the advantages and disadvantages of specific housing and management features for the welfare of farm animals. The idea of creating an index system for welfare assessment originates from a concept of Bartussek (1985), proposing a Tier-Gerechtheits-Index (TGI, translated as animal needs index) in the context of a state directive for intensive animal housing legislation in Austria. The concept has been further developed leading to the TGI35L/2000 (Bartussek 1999, 2000). The TGI35l/2000 is a method assessing the impact of the housing system on animal welfare of cattle, pigs and poultry mainly for organic production. Selected aspects of the animals' environment and farm management are assessed and scored, the higher the score the better the welfare. The scores are then summarized to give an overall welfare score. The name TGI35 comes from the fact that the maximum score possible was originally 35. The L (long version) comes from the fact that the initial version of the TGI was reexamined, several criteria were added (30 to 40 indicators in total depending on the husbandry) and the maximum score possible went to 45.5 (the lowest score possible is -9). In this study the TGI35L/2000 will be referred as TGI. The TGI is the current method used in controlling organic farms in Austria. The TGI is an easy, fast and repeaTable method to assess animal welfare on-farm. The TGI200 is another example of on farm welfare assessment (Sundrum *et al* 1994; Sundrum 1997). It is similar to the TGI but goes beyond certification and provides advises to farmers. More animal-based indicators are included and the maximum score possible is 200. Others methods utilized mainly animal-based (Capdeville and Veissier 2001) indicators, these methods a re more accurate but not practical for on-farm assessment due to the length of time needed to complete the inspection.

In the last decades European agriculture has changed significantly with increased mechanization and an increase in the number of part-time farmers, so that the time spent by the stockperson in contact with animals is thereby reduced (Rushen *et al,* 1999). These factors and the number of animals that are managed by a stockperson have been reported to influence human-animal interactions creating welfare (animals) and safety issues (animals and humans) (Hemsworth 2003). The welfare, health and management of farm animals are relevant concerns that need to be considered in order to increase consumer acceptance of animal production in the future. Declining farm incomes have pushed farmers out of full-time farming so that 60% of farmers are now operating on a part-time basis in Ireland (Connolly *et al* 2005). There is currently no scientific data available on the status of animal welfare on beef suckler farms in Ireland. A method derived from that of Bartussek *et al* (2000) was modified and was used to assess animal welfare at farm level.

Therefore, the objectives of the present study were to (i) evaluate animal welfare of Irish beef suckler herds using an AWI derived from the TGI (Bartussek *et al* 2000); (ii) investigate the impact of the activity of the farmer (full time or part time), the interest of the farmer and the number of animals on the AWI; and (iii) compare the results obtained with the AWI to those obtained with the TGI. A further objective was to analyze how the AWI is influenced by the indicators assessed and if different classes of farms in relation to housing and welfare could be identified.

2.2 Material and methods

Farm selection

The number of visited farms per county ranged from 13 to 20. The counties that were included in the study were: Cavan, Clare, Donegal, Kerry, Kildare, Laois, Leitrim, Limerick, Louth, Mayo, Meath, Roscommon and Tipperary. The Agricultural Officer (head of advisory centre) of the national agriculture research and extension organization (Teagasc) for each county in Ireland was contacted to identify suckler beef farms and selected farms close to each others in order to ease the journey of the assessors. The selected farmers were then contacted by their local adviser to know if they allowed their farm to be inspected. A total of 196 farms were visited and data for each indicator were collected for each farm.

Initial assessment

Five farms (not included in the study) were selected in an initial study to test the repeatability and to familiarize the two assessors with scoring of the indicators for use in the main study. Each of the 196 farms in the main study was assessed by the same two assessors.

Farm inspections

One hundred and ninety-six farms were visited to assess the AWI. Farms were visited from March 2006 to April 2007; once during the winter housing period and again at grass during spring. Three indicators of the farm size (number of hectares, number of cows, number of animals in the herd (cows, calves, heifers, bulls) and the working status of the farmer: part-time or full-time. By full time farmers it is implied that they required more than 0.75 labour units to operate) were collected (but not included in the scoring system). Two distracter objectives were given to the farmers to ensure they were naïve to the on-farm assessments; 1) to evaluate meal and silage quality and 2) to collect information on the efficiency of vaccines and antibiotics and anthelminthics.

In March 2009, a second visit of 23 arms out of the 196 was made to collect data using the AWI and the TGI at the same time in order to compare the AWI with the TGI. The selected farms were located in Meath and Clare counties because it was necessary to collect data at housing and that, at this period, most of the farms of other counties already turned out to grass. The farms were firstly assessed with the AWI then with the TGI by both operators for each method.

TGI

Draeger hand pumps and Draeger tubes measuring CO_2 and NH_3 levels were used to collect data at ground level (Nh3 emitted by slurry and CO2 heavier than the air)on the 23 farms using the TGI. An anemometer was used to collect data for the air flow was the flow was likely to be the most important (i.e. along adjacent walls from the shelter's door). Data were collected using the original indicators TGI (Bartussek *et al* 2000) (Table 1).

AWI indicators

The majority of the indicators listed in the TGI were unchanged, while scores for some indicators were adapted or modified to suit Irish conditions. New indicators were added. Some irrelevant indicators from the TGI were not used. The nature of the changes is explained in the definitions of the indicators below and summarized in Table 1.

The AWI grouped 33 indicators into five categories: "locomotion"; five indicators (Table 2), "social interactions"; seven indicators (Table 3), "flooring"; five indicators (Table 4), "environment"; seven indicators (Table 5) and "Stockpersonship"; nine indicators (Table 6). The higher the scores, the better were the conditions regarding animal welfare. The minimum score on the AWI was -11.5; the maximum was 46, giving a range of 57.5 points. Using the overall score allows compensating for poor conditions in one category, by better scores in another one, for example a lower score in the "locomotion" category could be compensated by a better score in the "environment" category. However, a check of the minimal requirements was performed before scoring a farm Minimum requirements were checked by the two operators and included the feeding (animals needed to be fed everyday and in sufficient quantity), drinking (animals needed constant water supply) and minimum space allowance described in the EU scientific report about the welfare of cattle kept for beef production (2001). If one of the minimal requirements was not achieved then the AWI was considered as "inadequate" without scoring any of the indicators.

Definitions of indicators and scores

Most of the indicators were directly taken from the TGI (Bartussek *et al* 2000) but were modified to meet the conditions of the study. The summary of the nature of changes is listed in Table1. In the "locomotion" category, the sub-indicators "slats", indicators "injurious protrusions", "outdoors access" and "ease of locomotion" were new indicators added for the AWI. "Lying down and rising" indicator from the TGI was modified and renamed in "ease of movement" in the AWI. The TGI only assesses the ease of lying down/rising in the lying area whereas the AWI includes lying down, rising and ease of movements in the pen. The "outdoors exercise" and "pasture/alpine pasture" indicators from the TGI were regrouped into the "grazing time indicator" in the AWI (Table 2). In the "social interactions category", the "calving method", "weaning method" and "rest area" and "outdoors access" indicators were new indicators added for the AWI. The "social structure of the herd" and "integration of followers" indicators from the TGI were regrouped in the "age/group mixing" indicator in the AWI. "Outdoors exercise" and "pasture/alpine pasture" indicators from the TGI were regrouped in the "grazing time" indicator (Table 3)". In the "flooring" category", indicators "resilience of floor" from the TGI was renamed "type of floor". The "Slipperiness of floor" from the TGI depends on the nature of the flooring and of its cleanliness; this indicator was not used in the AWI. "Cleanliness of lying area" from the TGI was modified in "yard cleanliness" in the AWI. "Slipperiness of outdoors area" was not used in the AWI; "yard type of floor" was used instead. "Floor condition of exercise area" from the TGI was modified in "yard cleanliness" in the AWI. "Pasture/alpine pasture" from the TGI was modified in "grassland" in the AWI (Table 4). In the "environment" category, the "artificial light" was a new indicator added for the AWI. The "air quality and flow" indicator from the TGI was not used but replaced by "side openings" and "condensation" indicators. The "days outside per year" and "hours outside per day" indicators from the TGI were modified and renamed "grazing time" in the AWI (Table 5). In the "stockpersonship" category, the indicators "background" and "level of interest of the farmer" new indicators added for the AWI. The indicator "cleanliness of pens, feeding/drinking areas" from the TGI was modified (Table 6) to give 3 indicators: "cleanliness of feed", "cleanliness of troughs" and "cleanliness of outdoors troughs". The parameter "technopathies" was not in the AWI but was assessed by the "injurious protrusions" indicator of the "locomotion" category. The indicator "state of hooves" in the TGI was not used and the indicator "lameness" was used instead in the AWI. "The indicator "animal health" from the TGI was renamed "diseases". When different conditions were observed for an indicator, the worst 25% (Bartussek 2000) were used for scoring. For example, if the flooring was dirtier in some areas than others, the dirtiest 25% was used for rating. When scoring the cleanliness of the animals, the 25% dirtiest were observed.

Locomotion

Space allowance

The space allowance was calculated by dividing the total area available in an animal pen by the total Animal Weight Unit (AWU) (one AWU represents 500kg of live body weight). The space allowance score was assigned to the pens with the smallest space allowance ratio in the herd. The size of the pens was measured with a rule or asked to the farmer when it was difficult to measure. The average weight of finished animals, the maximum number of animals in the herd during the year and the maximum number of animals in each pen were asked to the farmer, the first one to calculate the AWUs and the second one to calculate the space allowance then the ratio (m^2 /AWU) is the lowest. When animals were fed *ad libitum*, the animals were able to have their head outside the pen when eating at the feeding rack increasing the available area. In that case, the area represented by the length of the feeding space multiplied by 0.7 (extra space out of the pen when eating) was added to the available area. In the case of cubicles, half of the surface they represented was included because movement of the animals is restricted in cubicles (See Bartussek *et al* 2000). In the case of slatted floor facilities (more susceptible to engender hooves and/or limbs problems in the long term), the maximum score was one (according to the behavioural results of Hickey *et al* 2003). In the case of loose housing or tether systems, the scores were not modified from the TGI because it already covers these types of flooring (Table 2). Only one score is assigned to the space allowance and if, for example, two type of husbandry were found in one farm, such as slatted floors and loose housing, two AWI/TGI had to be calculated.

Outdoor yard access

This included yards that provided at least $3m^2$/AWU. The maximum score of 2 was assigned if the animals had full access to the yard at anytime. The maximum score for that indicator in the TGI is 3 because the number of hours/day the cows have access to an exercise yard has to be measured. Here the maximum score was 2 because the husbandry systems are different in Ireland; cows usually don't have access to outside yards due to anti-pollution regulations. The size of the outdoors yards (when present on–farm) was never bigger than $5m^2$ and in the TGI, only yards of at least $5m^2$ are scored. For that reason the maximum point that could be assigned in the AWI for the outdoors access indicator was dropped to 2. A score of 1 was assigned if the path to the outside yard hampered the animals exiting. A score of zero was assigned if the animals did not have access to an outside yard.

Injurious protrusions

If no injurious protrusions were noticed the score was unchanged. A score of -0.5 was assigned to the indicator if injurious protrusions that were likely to harm the animals were found. Partition, bars and other parts of the pen that could cause clinical symptoms that indicated deviation from the normal and the clinical symptoms on the animals were also examined. A particular attention was given to the nape of the neck, wither, coxal tuberosity, hair coat and joints to detect symptoms.

Ease of locomotion
A score of 1 was assigned if the shape of the pens allowed the animals to walk with no restriction in the pen and that they could stand up or lye down normally. If the shape was hampering the animals in their locomotion (i.e. if an animal moving from one point of the pen to another had to stop moving because of another animal or because of a part of the pen) or lying down/ standing up (low and repetitive head swinging, rocking back and forth etc.) a score of 0 was assigned. A score of -0.5 was assigned if the shape of the pens was restraining the animals' movements to a critical point (animals that could not move much or that could not move at all) and they had extreme difficulties to stand up or lye down..

Grazing time per year
The indicator "pasture/ alpine pasture" from the TGI was not applicable in the conditions of the study because there is no alpine/ mountain landscapes in Ireland. The indictor was renamed "grazing time per year" and the same scoring system than the TGI was used. A minimum score of 1 was assigned if the animals stayed more than 50 days per year at grass. A maximum score of 3 was assigned if the animals stayed more than 270 days at grass per year.

Social interactions
This category represents the level of social interactions using environmental-based indicators.

Space allowance
The same indicators were used as was outlined for the "locomotion" category.

Age of the animals/ groups mixing
This indicator was scored using the same scoring system than the TGI. Family herds consisted of suckler cows with male and female calves, heifers and steers born in the herd and/or bulls in the same pen or if the bulls were in an adjacent separated pen. That reflects the natural herd structure (Bartussek 2000, Warton 1957). A score 1 was assigned if the bulls were housed in separate pens and could not be seen by the other animals (or if the farmer was using artificial insemination and there was no bull on-farm) and/or the animals were grouped regarding their age, thus animals of the same age in a pen, with no regrouping. A score of 0 was assigned if minimal regrouping occurred during the year or if between 10 to 50% of buying-in occurred during the year. If frequent mixing and/or significant gap in the age (of animals that were not from the same family/ were issued from different sources) of the cattle of one same pen occurred and if more than 50% of animals were bought each year then the score was -0.5.

Rest area
Cubicles allow animals to rest in a private space and avoid negative interactions with more dominant animals (Bartussek 2000). The presence of cubicles, straw resting areas or yard used for rest (if the yard flooring was concrete the score was 0) was scored 0.5; their absence was scored 0.

Calving method
Wild cows, like other ungulates, often calve close to their group or move away from the group but always at a distance where they still can see the rest of the group. A score of 1 was assigned if the cows were allowed to calve in a separate pen from the rest of the animals, but within visual contact of their peers. A score of 0 was assigned if no visual contact was possible. If the cows had to calve in the pen amongst other animals, a score of -0.5 was assigned.

Weaning method
In nature, calves get independence gradually by drinking less and less milk from the dam and by foraging more and more. A score of 1 was assigned if the weaning process was gradual and calves had visual contact with the dams during weaning because it reflects the natural behaviour of the animals. A score of 0 was assigned if the weaning was abrupt. If no visual contact was possible the score assigned was -0.5.

Grazing time per year
The score for grazing time per year was similar to the "locomotion" category score except that the maximum score that could be assigned was 2.5 (Bartussek 2000).

Outside yard access
The same parameters were used to score the outside yard access as the ones used for the “locomotion” category except that a maximum score of 1 was assigned for the best conditions, 0.5 for average conditions and 0 if no yard access was available.

Flooring
Type of floor, cleanliness of floor
The score assigned to the floor type is shown in Table 4. If several types of flooring were present in the pen, the worst one was used for scoring. The dirtiest 25% of the area was used to score the cleanliness of the floor.

Outside yard; type of yard floor and yard floor cleanliness
The same parameters to those used to score the type of floor were also used to score this indicator.

Grassland
The “paddock size”, “frequency of new paddock”, “type of fencing”, “conditions of alleys and gaps”, “the presence of shelters for the animals” and the “grass management” were evaluated and used as sub-indicators to give a “grassland” score. The “paddock size” and “frequency of new paddock” were evaluated relatively to the size of the herd and were subjective parameters. The type of fencing was considered good if it was a electric wire or a stone wall; it was considered average or bad if barbwire was used and depending on conditions of the barbwire (for example rotten barbwire or presented in a way likely to harm the animals). The condition of alleys and gaps were considered “good” if they were not likely to harm the animals (for example flat or slightly sloppy terrain of grass). They were considered “average” if some items in the landscape could harm the animal (more sloppy surface or more rocky terrain) They were considered as bad if the slopes were too important and if the terrain was very likely to harm the animals (rocks, gaps, slippery surfaces). The presence of a shelter for the animals was considered good as it can protect them from wind or rain. In case of a natural shelter such as trees that could be used by the animal for sheltering the indicator was considered average. If no shelter could be used and the animals had to undergo wing and rain with no possibilities of sheltering the indicator was considered as bad. The farmer was asked at what height he was cutting the grass, how many toppings a year he was doing and how often he was performing a reseed. Depending on his answers and the number of the animals, the assessors discussed and agreed on a scoring “good”, “average” and “bad” to evaluate the grassland management. The maximum score was assigned if more than four sub-indicators were assigned “good”. A score of 0 was applied if 2 to 4 of these sub-indicators were assigned “good”. If less than 2 indicators were assigned “good” or if one indicator was considered as poor, a negative score of -0.5 was assigned.

Environment

Natural light,
Open fronted housing represented buildings with an area of at least 0.45m^2/AWU to unrestricted access to open air. The minimum height of these opening had to be 1m. Open fronted area represented the best light conditions for the animals (Bartussek 2000). If the building was not open fronted, the assessment of the natural light indicator that was directed at the animals was performed using the percentage of windows areas relative to the floor area was measured (0% was considered as very dark (no natural light); 15% and more as very light), the position of the open areas with access to direct daylight, on walls and/or roof and the cleanliness of the windows had to be taken into account to score the natural light.

Artificial light
The number of lights and their strength relative to the floor area of the building were used to assess the artificial light indicator. If no artificial light was present, the score assigned was -0.5. During winter there is not much light during the day, especially going up the north, and farmers often have to work during the dark. No light when working in darkness is stressful for the animals and can be harmful for them and the stockperson. In case of neons or CFLs if less than one light per 5m^2 was present the score assigned was 0. If one to 1.5 light per 5m^2 were present the score was 0.5; 1 was assigned if between 1.6 to 2 lights per m^2 were present and 1.5 was assigned if more than 2 lights per 5m^2 were present. In case of halogen lights 0 was assigned of there was less than one lamp per 15m^2, 0.5 if there was between 1 and 1.5 lamp per 15 m^2, 1 if there was between 1.6 to 2 lamps per 15 m^2 and 1.5 if there was more than 2 lamps per m^2. This was based on an average of 50W for

neons and CFL and 500W for halogen lamps but it was not possible to know the exact light intensity received by the animals.

Side openings
Side openings contribute to a good airflow and thus air renewal in the buildings. The presence of side openings on the walls of the housing facilities was scored as 0.5, the absence of the latter was score 0.

Draughts
A moist finger was used to assess the presence of draughts at different point of the housing. Area that are likely to get draughts (along the walls adjacent to doors, gates and openings) where examined for the AWI (Bartussek 2000). If no draughts could be found and the building's structure was designed to reduce them, a maximum score of 1 was assigned. The minimum score of -0.5 was assigned if draughts could be found in only one spot of the building and that the shape of the building allowed frequent draughts.
To assess this indicator for the TGI (data were collected on 23 farms). The presence of draughts was checked using an anemometer (direct reading of wind speed) and the Draeger tubes for CO_2 levels (indirect measure that is representative of the air exchange between the inside of the building and the exterior; Bartussek 2000). A draught was considered if the speed of the air flow was greater than 0.2m/s.

Noise levels
The building was checked for presence of fans/ventilation system to assess the noise levels. A score of 1 was assigned if the noise levels on farm were very low that is to say if there was natural ventilation with no technical help. The minimum score of -0.5 was applied in the case of a very noisy environment (ventilation made by fans that start to be irritating for the human ear, that is to say around 70 dB). It was no possible to measure the intensity of noise with technical equipment.

Condensation
It was assumed that the air could not be to dry in the Irish conditions. This parameter was assessed subjectively because no technical equipment was available to measure the hygrometry of the housing. A maximum score of 0.5 was assigned if no condensation or humidity was felt on the naked forearms of the assessors or if the building was open fronted. A score of 0 was assigned if a sensation of humidity could be slightly felt on the forearm of the assessors (light feeling of air humidity). A score of -0.5 was assigned if the humidity was clearly felt (strong feeling of air humidity) on the forearm of the assessor if wetness or condensation droplets were noticed on the wall and/or roof.

Grazing time per year
The maximum score assigned was 2.5 instead of 3 (for locomotion and social interactions categories) (Bartussek 2000).

Stockpersonship
These indicators reflect how the farmer tries to provide a well-managed housing environment

Cleanliness of troughs/outdoor troughs.
The troughs were considered clean when the water was clear, no algae could be seen in the water and no mud/slurry was present on them. Troughs were considered medium when the water was clear but small amount of algae could be found and/or few spots of mud/slurry of less than 2 cm of diameters were present on them. They were considered insufficient if the water started to be blurred but it was still possible to see through it, if the amount of algae was preponderant and if many spots of less than 2cm of diameter of mud/slurry were present on them. They were considered soiled if the water was blurred and it was not possible to see through it, if algae colonized the troughs and if mud/slurry covered the troughs or many spots of more than 2 cm of diameter were found.

Feed cleanliness
Clean feeding spaces were assigned with the maximum score. If the presence of old feed slurry/mud was found at the feeding place then the maximum score could not be assigned, depending of the amount of old feed/slurry/mud different scores were assigned from 0.5 to -0.5. The score was assigned after the two assessors discussed a rank foe the cleanliness.

Conditions of the equipment

The maximum score was assigned when the equipment was recent (less than 5 years) and safe. The minimum score was assigned if critical defects could be found and that could be dangerous for the stockperson and/or cattle or if no handling system was present on-farm.

Cleanliness of animals

Animals were considered as clean if they were covered with less than 10% of slurry/mud, they, medium between 11 and 20% and soiled over 20%.

Lameness

The presence of lameness in cows was reported by the farmer. A maximum score of 0.5 was assigned if less than 5% of lame cows was reported; a score of 0 if between 5 and 9% of lame cows and -0.5 if 10% or more of lame cows.

Diseases

It was not possible to investigate the health records of the animals. A distracter objective was given to the farmer. They were told the study's target was to evaluate the efficiency of vaccines, antibiotics and anthelminthincs and they were asked for what diseases they were vaccinating against, which anthelminthics they were using, what antibiotics for treatment of diseases they used, if they were dosing and if they noticed the diseases or symptoms after the treatments in the last year. No information about the occurrence of a disease in a herd could be gathered.

The list of diseases and symptoms consisted of: respiratory diseases, scours, worms, parasites (mild diseases), BVD, BRD, Johne's disease, tuberculosis, leptospirosis and black leg (severe diseases). A maximum score of 1 was assigned if no diseases were reported. The score was 0.5 if up to 2 mild diseases or symptoms were reported. A score of 0 was assigned if the presence of one or two severe diseases or more than two mild diseases reported. A negative score of -0.5 was assigned if the presence of more than 2 severe diseases was reported or 4 mild diseases were reported.

Background

Farmers having an agricultural background tend to detect problems faster than the one who don't. Stockpersons with an agricultural background (family farming) were assigned a score of 1 while those with no agricultural background received a score of 0.

Interest of the stockperson

The interest of the farmer was evaluated using five questions. The farmers were asked "How much time do you spend with the animals?" "Would you spend more time if you could?" "How do you consider farming nowadays?" "What training did you receive?" "Were you happy with the training?" This parameter was very subjective as it was possible that the farmers could have been dishonest and it was also relative to the operators. The scores were 1 if the answers were going toward a good interest, 0.5 if the interest seemed to be average -0.5 if the interest seemed to be low.

AWI score

For each category, the indicators were evaluated and the farm was scored. The score for each indicator within a category was summated to give a category score. The category scores were then summated to give an AWI. The minimum score possible was -11.5 and the maximum score was 46, with a range of 57.5 points. The raw score was transformed into a relative score.

AWI = (Locomotion score + Social interactions score + Flooring score + Environment score + Stockpersonship score + 11.5)*100/57.5.

Farms were rated with ranks. The same ranking scale was used than the one from the TGI. The animal welfare was considered as "inadequate" (IA) between 0 to 15% of the AWI maximum score, "adequate" (A) from 16 to 30%, "satisfactory" (S) from 31 to 50%, "good" (G) from 51 to 60%, "very good" (VG) from 61 to 75 and "excellent" (E) above 75% (Bartussek 2000).

Statistical analysis

The AWI and the category scores were tested for normality using a Shapiro-Wilk test (Genstat 11th edition VSD UK). The Student t-test for unpaired samples (Genstat 11th edition) was performed to evaluate statistical differences between the AWI and the TGI 35L/2000 and the differences in AWI scores between farmers' status (full or part time). The Student test for paired samples was used to investigate the differences between the AWIs of the first and second visits. Mann-Whitney tests were performed to determine the differences in individual indicator scores (not continuous variable) and

Spearman's rank correlations were performed to identify the correlations of the number of animals and the interest of the farmers with the other indicators of the AWI. A Principal Component Analysis (PCA) was performed (SPAD 6.5) with the different indicators of the AWI. When two indicators were highly correlated (r value > 0.7), the indicator with the least other correlations was selected, then a second run of the PCA was conducted including the illustrative data as in Mazurek *et al,* 2007. The PCA was performed using DECISIA SPAD 6.5 software using the COPRI procedure and a hierarchical classification was determined using the PARTI-DECLA procedure. Twenty-seven variables were entered as active continuous. Two illustrative continuous variables were also added (Table 6).

2.3 Results

Animal diets during housing

Representative silage and meal samples were collected from the individual farms during the winter period. *In vitro* DM Digestibility of silage and concentrate feed samples were determined using the method of Tilley and Terry (1963). Animals had free access to grass silage (Mean *in vitro* DM digestibility = 603.8 g/kg DM (±10.6 s.e.); mean crude protein (CP) = 117.4 g/kg DM (±2.7 s.e.)) supplemented with concentrate feed (*in vitro* ADF = 132.3±5.4 (s.e.) g/kg, CP = 155.4g/kg DM ±3.2 (s.e)).

Farms status

Sixty-six percent of the interviewed farmers were full time (FT) farmers while 34% were part time (PT) (Table 3). The total number of cattle per farm ranged from 15 to 1000 with a mean of 131 ± 9.9 (s.d.). Part time farmers had a mean of 80 ± 7.7 (s.d.) animals and ranged between 17 and 370 animals per farm while full time farmers had 160 ± 13.5 (s.d.) animals per farm with a range comprised between 15 and 1000 animals (Figure 1). Medians were respectively 61 and 120 animals. Part time farmers (PT) owned a mean of 47 hectares while full time farmers (FT) owned a mean of 76 hectares (P < 0.001).

AWI distribution

The score for each category was calculated (Table 7). The AWI ranged from 54% to 83% of the maximum score with a mean of 65% (s.d. = 7%) (Figure 2). The mean "locomotion" score was 52%. The mean "social interactions" score was 50%. The mean "flooring" score was 49%. The mean "light and air" score was 88%. The mean for the "stockpersonship" score was 87%. The overall AWI ranged from "satisfactory" to "excellent" with a large majority (70 %) of the farms rated as "Very Good" or "Excellent". The categorization of welfare status (inadequate, adequate, good, very good and excellent) is shown in Figure 3. No farm was scored as "inadequate" or "adequate". One farm was graded as "satisfactory", 58 farms were graded as "good", 118 farms were scored as "very good" and 17 farms were scored as "excellent".

No differences were observed for the AWI between full and part time farmers. The "social interactions" score had an average of 48% for full time farmers and 52% for part time farmers. They were considered as "satisfactory" for the full time farmers and "good" for part time ones." Part time farmers had better "social interaction" category scores (P = 0.001). For individual indicators, full time farmers had better scores for cleanliness (P = 0.03) of the animals and had less lame cows (P = 0.01). They had a tendency to have better "level of interest" scores (P = 0.052). Part time farmers had better scores for the "grouping" (P<0.001) and "weaning" (P = 0.03) indicators (Table 8).

Statistical correlations

The number of animals was significantly correlated with the "health" score (R_s = -0.8, P < 0.001), the "social interactions" category score (R_s = -0.35, P < 0.001), the "age/ group mixing" score (R_s = -0.32, P < 0.001), the "weaning method" score (R_s = -0.23, P < 0.001) and the AWI score (R_s = -0.21, P = 0.001). Lower correlations between the number of animals and other indicators were also found and presented in Table 10. The interest of the farmer was correlated with the "stockpersonship" category score (R_s = 0.67, P < 0.001), the "feeding space cleanliness" score(R_s = 0.62, P = 0.012), the "floor cleanliness" score (R_s = 0.47, P = 0.01), the "outdoor water cleanliness" score (R_s = 0.44, P = 0.001), the "lameness" (a higher score indicates less lameness) score (R_s = 0.0.43, P < 0.001), the AWI score (R_s = 0.42, P < 0.001) and the "health" score (R_s = 0.42, P = 0.023). Lower correlations between the interest of the farmer and other indicators were also found and presented in Table 10.

AWI/ TGI comparison

A significant difference (P < 0.001) was found between the two indices. The TGI scores were lower (mean of 59 ± 7% (s.d.)) than the AWI scores (mean of 65 ± 6% (s.d.)). No difference (P > 0.05) was found for the locomotion category. A significant difference was found for the social category with mean scores of 37 **±** 9% (s.d.) for the TGI and 53 ± 8% (s.d.) for the AWI, respectively. No significant difference (P > 0.05) was found between the two indices for the flooring category. A significant difference was found for the environment category with mean scores of 63 ± 11% (s.d.) for the TGI and 88 ±7% for the AWI. A significant difference (P < 0.001) was found for the stockpersonship category with 83 ± 6% (s.d.) for the TGI and 92 ± 9% (s.d.) for the AWI. Two farms went from "very good" rating with the TGI to "excellent" rating with the AWI. Five farms went from "good" rating with the TGI to "very good" rating with the AWI. Eight farms went from "satisfactory" rating with the TGI to "good" rating with the AWI.

AWI stability

No significant difference (P > 0.05) was found between the scores of the first visit and second visit. A high correlation was found between the scores (r = 0.86, P< 0.001) from the two visits.

Principal component analysis (PCA) and hierarchical classification

The PCA showed that 20% of the total variation of the information given by the indicators was explained by the first two axes (synthetic variables or factors). The first factor was described by the stockpersonship and represented 11% of the variation. The second factor was described by the animals' health and probabilities of injuries (the indicators correlated to this factor were: "health", "ease of locomotion", and "injurious protrusions") and represented 9% of the variation. The first 10 factors (61% of the total variation) were used to calculate the classes for the hierarchical classification. Three classes were found within the hierarchical classification. The first class corresponded to farms with the best mean AWI (66% as an average). The mean number of animals of this class was the same as the general mean. The class regrouped clean farms with a good environment and with a higher interest of the farmer than the average (0.82 against 0.71 for the general mean). This class represented 130 farms. The second class corresponded to farms that had a number of animals equal to the general mean. The AWI was the second in rank with 62% as an average. The class was characterized by better floor type and better natural light than the general mean but more injurious protrusions and worse ease of locomotion than the general means. They were also characterized with more diseases than the average. The interest of the farmer was lower than the general mean with 0.51 against 0.71 for the general mean. This class corresponded to 59 farms. The third class corresponded to five farms that had a lower AWI than the general mean (56%). It was correlated with poorer equipment, lower stockpersonship resulting in dirtier conditions and more diseases than the general mean. The mean number of animals for this class was similar to the general mean (Figure 4).

2.4 Discussion

The main objective of the study was to evaluate animal welfare on farm of Irish beef suckler herds. A second objective of the study was to compare the results of the AWI with the results of the TGI. Although animal based indicators are more likely to be a better assessment of animal welfare than environment-based indicators (Keeling & Bock 2007), they are not practical to evaluate on farm because they are demanding in time and labour from the farmer. Because of these limitations, the indicators that were measured in the present study were mainly environmentally based. Animal based and health indicators were also included. It was not possible to measure some of the indicators and data had to be collected qualitatively instead.

The AWI used indicators that were in the original TGI (Bartussek 2000), some of these indicators were modified and new indicators were also for use on Irish beef suckler farms. Some indicators from the TGI were not used, for example, the levels of CO_2 and NH_3 in the animal housing. It is reported in the literature that the background of the farmer is important in the detection of welfare problems (Lensink *et al* 2001), therefore the indicator "background" was included in the AWI assessments. The importance of the interest of the farmer in the management of animals well being is well documented (Hemsworth, 2003), thus the "level of interest" indicator was included in the AWI. The presence of cubicles, the calving and the weaning method were also used in the AWI and were not included in the TGI. Cubicles are not usually found in Beef husbandries but in Ireland it happens that farmers turn from dairy to beef and keep the old settings.

In agreement with the findings of Bartussek (1999), it was possible to define an on-farm welfare score with the AWI. A large percentage (71%) of farmers were participants in the Rural Environment Protection Scheme (REPS). The latter is a scheme designed to reward farmers for carrying out their farming activities in an environmentally friendly manner and to progressively improve environmental practices (http://www.agriculture.gov.ie).

Two thirds of farms visited were managed by full time farmers and one third by part-time farmers. By full-time farmers it is implied that they require more than 0.75 labour units to operate (Connolly *et al* 2005). The figures obtained in the present study are different from the National statistics in Ireland where part-time farmers are more numerous than full time farmers (62 vs. 38% in 2005, Connolly *et al* 2005). However these statistics related to all types of farms and not specifically beef suckler farms. Furthermore, in the present study, no difference was found between the full-time and part-time farmers regarding the AWI. Regarding the number of animals per farm, the upper quartile was 150 animals and the median was 100 animals. This was in accordance with the trend of the slightly increasing number of animals per farm reported in the Teagasc National Survey (Connolly *et al* 2005). Part-time farmers had a mean number of animals that was half of that of the full-time farmers. It was not possible to know if the time allocated to the animals was equal or not.
The "locomotion" score was reduced due to the fact that locomotion was limited during housing as most of the housing types did not have access to an outside yard during winter and cattle were often intensively housed on slatted floor. However, it is important to note that under Irish conditions outdoor yards are discouraged for environmental protection reasons, e.g. the REPS scheme (department of Agriculture, Fisheries and Food website, 2009), specifies that farmers should not have an outdoor yard in order to protect the environment (pollution, landscape protection). This explains why most farms do not have access to outdoor yards. However this is counterbalanced by the fact that the animals remain a longer time at grass during the year as explained further. The "flooring" score was lower because most of the farmers use slatted floor sheds rather than loose housing. The "environment" and "stockpersonship" category had scores reaching 88 and 87% respectively, placing these categories as "excellent" for both full-time and part- time farmers.

No difference was observed between the AWI for full-time and part-time farmers however part-time farmers had better "social interactions" category scores than full time farmers, due to better weaning and calving scores, the latter may be due to the lower number of animals being managed that allows the part-time farmers to meet better conditions. It was found that full-time farmers had cleaner animals and lower incidence of lameness. This could result from the fact that full-time farmers spend more time working on farm than part-time farmers. A tendency (P= 0.052) to have a better interest was also observed for full-time farmers.

The comparison with the TGI showed that the scores using the AWI were higher due to higher social interaction, environment and stockperson scores. It was not stated in the TGI, for the "locomotion" category, how to score housing systems that utilized concrete slatted floor sheds. This category was modified to allow the scoring of the space allowance indicator in Ireland where most of the animal housings have slatted floor facilities. The maximum score assigned for pasture in the TGI was 1.5 and 3 for access to outside yards. In the Austrian system cows go to grass for a short period (usually less than four months, Bartussek 2000) but would have daily exercises. In contrast, in seasonal grass based systems in Ireland, beef production systems typically comprise of a grazing season (usually eight months but it can happen that the grazing period is shorter when the weather conditions are bad) followed by an indoor winter period. In these systems, typically, the majority of calves are spring-born and they are allowed to continually nurse the dam at pasture until the end of the grazing season in autumn when they are weaned and generally housed indoors for a period up to five months (Drennan and McGee, 2008; 2009). It was necessary to adapt the indicator by modifying the maximum score assigned for the grass period from 1.5 in the TGI to 3 in the AWI. The "access to an outside yard" scores were changed from a maximum score of 3 (TGI) to a maximum of 2 in the AWI because the TGI was made to score cows that would not spend much time at grass and thus would need an exercise yard during housing. The indicators relative to time spent at grass (time grazing per year and grassland management) represent 9.5 points out of the 57.5. This represents 16.5% of the total AWI score. Therefore a stockperson that would not allow his animals to go to grass during the summer period could loose these points on the total AWI. Although the regulations, in Ireland, favor intensive breeding for environmental reason and thus not many access to outside yards are provided to the animals, greater AWI were possible due to the long time the animals stay at grass. Since the conditions in Ireland are different, the animals spend less time in housing they were not assigned the maximum score that was allocated for this indicator in the TGI. These changes didn't affect the final locomotion category score between the 2 indices. The "social interactions" category was modified as well. The management of young cattle is different in beef suckler herds in Ireland than in Austria so the indicator was not scored as an individual indicator (TGI) but was taken into account in the "grouping" indicator (AWI). Adapted scores for slatted floors, pasture and access to a yard were necessary. New indicators regarding the calving conditions and weaning methods were added. The scores were higher with the AWI because of the impossibility to score greater than 1.5 for pasture with the TGI (maximum score of 3 with the AWI) and because of the new added indicators. The flooring score was modified to take into account the type of floor of the yards and their cleanliness. In the TGI the type and cleanliness are mixed in the same indicator. Scoring the type and cleanliness of the floor and yard gives a more accurate description of the indicator. However no difference in scores was found between the TGI and the AWI for the "flooring" category. The environment category gave higher scores for the AWI. This results again in the fact that the TGI doesn't take into account the grazing period but the number of hours per day and days per year cows have access to an outdoor area. With a usual period of 4 months at housing (only two farms housed the cattle for six months) the Irish farms could not be scored greater than 1 for the number of days with outdoor access. The stockpersonship category scores were also higher for the AWI because new indicators were added. Indicators such as the "interest of the farmer" are important (Hemsworth 2003) and the background of the farmer has an impact on animal welfare (Lensink *et al* 2001). The PCA also showed the importance of the stockpersonship in the animals' welfare. The stockpersonship was the most discriminating factor. The second most discriminating factor was the probability of injury of the animals. The indicator "interest of farmer" was the most correlated indicator with the first factor (stockpersonship) resulting from the PCA. It was also the most correlated with the AWI .The hierarchical classification and the correlations showed that the interest of the farmer was one of the most important indicators to classify the farms. The cleanliness of pen, feeding area and drinking area were singly scored for a more accurate assessment. However this represents 4 points in the AWI and only 1 point in the TGI. Some collected indicators were also reported by the farmer like the presence of the different diseases or the percentage of lameness. It was not possible for the assessors to check the presence of lameness or diseases but health record books should be checked for more accuracy while rating these indicators. The severity of lameness should be taken into account too. The most reported diseases were parasites (worms, lice) and respiratory diseases (pneumonia). Very few cases of brucellosis, tubercular or bovine viral diarrhea were reported. Most of the scour problems were reported to occur in calves and were easily managed.

The correlations showed a strong association between the number of animals being managed on farm and the AWI. Indicators that were highly correlated include; the "social interactions" category notably the "grouping" indicator, the weaning method and calving (lower correlation).

The number of animals also has an impact on the number of diseases reported. A greater number of animals resulted in more different types of diseases reported on-farm, thereby reducing the health score. The number of animals had a negative correlation to the locomotion score and the noise score. The locomotion score was lower when more animals were raised supposing that the density of animals increases is bigger in larger farms. A negative correlation was found with the type of floor. This was influenced by the type of bedding available. In Ireland for example, there is insufficient straw available for animal bedding over the winter period and the cost is prohibitive. Straw is still used in small farms but generally is cost prohibitive on larger farms. Thus farmers tend to use slatted floor housing facilities on larger cattle farms. The straw bedding was usually used in farms with less than 80 animals in total but it was also used in on some larger farms (these farms also tended to have their own barley enterprise). Human-animal interactions (HAR) are a common feature of modern intensive farming systems and these interactions have been reported to have marked consequences on animal productivity and welfare (Hemsworth, 2003). Research has shown that we should not underestimate the role and impact of the stockperson on animal performance and welfare (Grignard *et al* 2001; Hemsworth 2003; de Passillé & Rushen 2005). Our study confirmed the importance of the farmer by his level of interest and was highly correlated with the stockpersonship score. A strong positive correlation was also found between the interest of the farmer and the AWI. More generally, a greater level of interest was linked with less lameness (reported by the farmer), a better cleanliness of the equipment, floor and of the animals, less diseases (reported by the farmer), better environment score and better artificial lights score due to better buildings, a better locomotion score, better weaning methods and better social interaction scores. The interest of the farmer was not correlated with the number of animals and this is in agreement with Hemsworth (2003). The level of interest of the farmer was assessed by a questionnaire. It has been reported that the attitude of the stockperson was also important for the animals' welfare (Lensink *et al* 2003; Waiblinger *et al* 2006). It was not possible to observe the farmer while working for reasons of timing, however, this is an indicator that should be included in future welfare assessments. If a farmer is interested but has wrong beliefs, this can have an impact on his animals (Lensink *et al* 2003). It would be of interest to analyze and understand the HAR in order to include relevant indicators that would be accurate and quick to assess. The number of diseases and the level of lameness should be taken into account for an accurate assessment of the animals' welfare. The 25% worst animals should be identified on-farm and used to rate the indicator of lameness if it is not possible to inspect all the animals. The assessment of lameness and observing the farmer at work would extend the time spent on farm during the inspection. Two inspections should be done: when the animals are at grass, when they are housed.

Three classes of farms were found. The classes were well separated within the first two axes. For the three classes, the interest of the farmer entered into the characterization of the classes (higher interest for the best scores and lower interest for the lowest scores). The number of animals for each class was not different from the general mean. In the third class, the conditions were poorer than the general ones and the level of interest of the farmer was significantly inferior than the general mean. The AWI was significantly lower than the general mean. This was in agreement with the literature (Hemsworth 2003; Lensink *et al* 2001).

The method showed that beef suckler herds in Ireland provide from "satisfactory" to "excellent" welfare of the animals. Regardless of whether the farmer worked full-time or part-time on farm, this did not affect the overall AWI scores while social interactions scores were greater for part time farmers. This study was based mainly on environmental indicators and limited animal-based indicators. Some indicators used were subjective and controlled experiment should be done to validate their scoring system and allow collecting measures to assess them. The PCA confirmed that the stockperson is an important factor in determining the AWI (11% of the total variation) more specifically, the interest of the farmer. Thus the stockperson needs to be taken into account when describing the welfare conditions of animals on-farm.

Animal welfare implications

The welfare, health and management of farm animals are important factors that need to be considered in order to maintain optimal animal welfare and increase consumer acceptance of animal production in the near future. The present study highlighted that the welfare status of Irish beef suckler farms seemed to be of a high standard and that there was no difference in animal welfare between full-time and part-time farmers. It was shown that the interest of the farmer and the number of animals on-farm are important factors that influence the overall animal welfare. The AWI was more suitable to the Irish conditions than the TGI. The AWI is an easy and quick method that could be used in countries with similar farm management as in Ireland but further research is needed to validate the assessment and the weight of some subjective parameters

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Table 1 **Structure of the TGI35L/2000 for young cattle, beef cattle and cows and nature of changes to obtain the AWI.**

Field of influence to be evaluated	Ethologic and hygienic arguments	Indicators assessed with theTGI35L/2000	Points (min-max)	Nature of changes	Indicators assessed with the AWI	Points (min-max)
I. Possibility of movement	Sufficient movement	Area per animal, m^2/500kg	0-3.0	U	Space allowance	0-3.0
	Normal behaviour at			A	Slatted floor	0-1.0[b]
	resting, lying rising,			A	Injurious protrusions	-0.5-0
	Five freedoms" according to the Brambell Report (Brambell, 1965)	Rising, lying down in loose housing	0-3.0	M	Shape of pen	-0.5-1.0
				A	Outdoors access	0-2.0
		Tied housing	0-2.0[b]	U	Tether systems	0.2.0[b]
		Outside exercise	1-3.0	R	Grazing time/outdoor access	0-1.0
		Alpine pasture/ pasture	0.5-1.5	R	Grazing time	1-3.0
II. Social contact	Agricultural animals are social	Area per animal, m2/500kg	0-3.0	U	Space allowance	0-3.0
	species	Social structure of herd	-0.5-2.0	R	Age/group mixing	
	Essential needs for	Integration of followers	-0.5-1.0	R	Age/group mixing	-0.5-2.0
				A	Calving method	-0.5-1
				A	Weaning method	-0.5-1
				A	Rest area	0-0.5
				A	Outdoor access	0-1.0
	species-specific social	Outside exercise	1.0-2.5	R	Grazing time/outdoor access	
	contact and behaviour	Alpine pasture/ pasture	0.5-1.5	R	Grazing time	0.5-2.5

[a] Slatted floor systems and tied housing are sub-indicators to assess the space allowance, are mutually exclusive and mutually exclusive with loose housing**.**

Table 1 Structure of the TGI35L/2000 for young cattle, beef cattle and cows and nature of changes to obtain the AWI. (continued)

Field of influence to be evaluated	Ethologic and hygienic arguments	Indicators assessed with theTGI35L/2000	Points (min- max)	Nature of changes	Indicators assessed with the AWI	Points (min-max)
III. Quality of flooring	Permanent contact,	Resilience of lying area	-0.5-2.5	U	Type of floor	
	Important effects on	Slipperiness of floor	-0.5-1.0	R	Type of floor	-0.5-2.5
	behaviour,	Cleanliness of lying area	-0.5-1.0	U	Cleanliness of floor	-0.5-1.0
	hygiene,	Slipperiness of outdoors area	-0.5-1.0	M	Yard type of floor	-0.5-2.5
	health and well-being	Floor condition exercise area	-0.5-1.5	M	Yard cleanliness	-0.5-1.0
		Alpine pasture. Pasture	0.5-1.0	M	Grassland	-0.5-1.0
IV. STable climate	Permanent contact,	Daylight in animal house	-0.5-2.0	U	Natural light	0-2.0
	Important effects on			A	Artificial light	0-1.5
	behaviour,	Air quality	-0.5-1.5	N		
	hygiene			A	Side openings	0-0.5
	health and well-being	Draughts within lying area	-0.5-1.0	U	Draughts	-0.5-1.0
				A	Condensation	-0.5-0.5
		Technical noise	-0.5-1.0	U	Noise	-0.5-1.0
		Days outside/ year	0.5-2.0	R	Grazing time	1.0-3.0
		Hours outside/ day	0.5-2.0	R	Grazing time	

A: indicator not included in the TGI35L/2000 and added for the AWI. M: indicator from the TGI35L/2000 that was modified in the AWI. N: indicator that was included in the TGI35L/2000 but not used in the AWI. R: indicators of the TGI35L/2000 that were regrouped in another indicator of the AWI. U: indicator identical to the TGI35L/2000.

Table 1 Structure of the TGI35L/2000 for young cattle, beef cattle and cows and nature of changes to obtain the AWI. (continued)

Field of influence to be evaluated	Ethologic and hygienic arguments	Indicators assessed with theTGI35L/2000	Points (min- max)	Nature of changes	Indicators assessed with the AWI	Points (min-max)
V. Care of stockperson	Correct and attentive care/ handling of animals has a balancing and compensating effect on behaviour, hygiene, health and well-being	Cleanliness of housing	-0.5-1.0	N		
				A	Troughs cleanliness	-0.5-1.0
				A	Outdoors troughs cleanliness	-0.5-1.0
				A	Feed cleanliness	-0.5-1.0
		State of technical equipment	-0.5-1	U	Equipment	-0.5-1.0
		State of coat hair	-0.5-1	R	Injurious protrusions	
		Cleanliness of animals	-0.5-0.5	U	Animal cleanliness	-0.5-0.5
		State of hooves	-0.5-1.5	M	Lameness	-0.5-1.0
		Technopathies	-0.5-1.5	R	Injurious protrusions	
		Animal health	-0.5-1.5	M	Health	-0.5-1.0
				A	Background	0-1.0
				A	Interest of farming	-0.5-1.0
Sum of points			-9.0-45.5			-11.5-46

[a] Technopathies are damage and injuries that are caused directly or indirectly by the construction. A: indicator not included in the TGI35L/2000 and added for the AWI. M: indicator from the TGI35L/2000 that was modified in the AWI. N: indicator that was included in the TGI35L/2000 but not used in the AWI. R: indicators of the TGI35L/2000 that were regrouped in another indicator of the AWI. U: indicator identical to the TGI35L/2000.

Table 2 **Indicators in the AWI of the "Locomotion" category, the definitions used for rating and their maximum individual score. In tether systems, the first figure refers to back and forth movements, the second to lateral movement.**

Locomotion							
Score	**a) Space allowance**			**b) Outdoor access**	**c) Injurious protrusions**	**d) ease of locomotion**	**e) Grazing time**
	Slats[3] (m^2/AWU)[2]	**Loose housing (m^2/AWU)**	**Tether systems Movement of tether (m)**				**(days per year)**
3		>7.5					>270
2.5		>6.5					>230
2		>5.5		Yes, all the time			>180
1.5		>4.5					>120
1	>3	>4	>0.6/0.4	Yes, partially		Easy locomotion	>50
0.5	2< X[1] < 3		>0.4/0.3				
0	<2	<4	<0.4/0.3	No	No	Partially restraining	
-0.5					Yes	Restraining	

[1] Represents the observed value between 2 and 3 m^2/AWU. [2]AWU means Animal Weight Unit; 1 AWU = 500kg liveweight. a) Refers to the space allowance; only one type of system is cored; if different systems are found on-farm, each system must be scored independently. b)I If animals have constant access to an outside yard and they can all be outside at the same time the maximum score is assigned, if the access is restricted and/ or not all animals can go out, the score of 1 is assigned. If there is no access to a yard the score assigned is 0. c) Refer to any part, partition and bars susceptible to harm the animals; the teguments of animals are also checked to detect any sign of deviation from the normality. d): If animals can move easily the maximum score is assigned; if the animals need to stop when moving and/or have difficulties to rise/lye down, the score assigned is. If the movements of the animals are very restrained and/or if they have extreme difficulties to rise/ lye down, the score assigned is -0.5. e) Total days spent at grass per year The total locomotion score equals to the sum of columns a),b),c),d) and e)

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Table 3 **Indicators in the AWI of the "Social interactions" category and the definitions used for rating and their maximum individual score.**

Score	a) Space allowance			b) Age/group mixing[1]	c) Rest areas	d) Calving method[3]	e) Weaning method[3]	f) Outdoor access	g) Grazing Time (days per year)
	Slats	Loose housing	Tether systems						
	(m^2/AWU)[2]	(m^2/AWU)[2]	Movement of tether (m)						
3		>7.5							
2.5		>6.5							>270
2		>5.5		Family herd					>230
1.5		>4.5		Herd without bull					>180
1				Same age		Separate pen	Visual contact	Yes	
	>3	>4	>0.6/0.4	No regroup		Visual contact	Gradual	All the time	>120
0.5	2< X[1]< 3		>0.4/0.3		Yes			Yes Partially	>50
0				Minimal Regroup/age mix		Separate pen	Visual contact		
	<2	<4	<0.4/0.3		No	No visual contact	Abrupt	No	
-0.5				Frequent Regroup/age mix		In pen with other animals	No visual Contact		

[1] Represents the observed value between 2 and 3 m^2/AWU. [2]AWU means Animal Weight Unit; 1 AWU = 500kg liveweight. a) Refers to the space allowance; only one type of system is cored; if different systems are found on-farm, each system must be scored independently b) Family herd consist in suckler cows with male and female calves, heifers and steers of the same family and integrated bulls, The total social interactions score equals to the sum of columns a), b), c), d) e), f) and g).

Table 4 Indicators in the AWI of the "Flooring" category, the definitions used for rating and their maximum individual score.

			Flooring		
Score	**a) Type of floor**	**b) Cleanliness of floor**	**c) Yard type of floor**	**d) Yard cleanliness**	**e) Grassland**
3					
2.5	Straw >60mm		Straw >60mm		
2	Straw 30-60 mm		Straw 30-60 mm		
1.5	Woodchip/peat		Woodchip/peat		
1	Mats	Clean	Mats	Clean	Good conditions
0.5	Softer slats	Medium	Softer slats	Medium	
0	Concrete slats	Soiled	Concrete slats	Soiled	Average conditions
-0.5	Concrete	Very soiled	Concrete	Very soiled	Poor conditions

a) and c) Softer slats refers to slats softer than concrete (for example wooden slats). b) and d): Clean: no slurry/mud can be found on the pen (100 to 80% for the straw or woodchip/slurry ratio); medium: not more than 3 spots of slurry/mud can be found in the pen for slatted floors (79 to 60% for the straw or woodchip/slurry ratio) soiled :more than 3 spots of slurry can be found in the pen (59 to 40% for the straw or woodchip/slurry ratio); very soiled: the pen is covered with slurry/mud (less than 40% for the straw or woodchip/slurry ratio). e) Score assigned after checking the paddock size and frequency of new paddock regarding to the size of the herd, boundaries, conditions of alleys and gaps, number of topping per year and frequency of grass reseed and presence of shelters. The total category score equals to the sum of columns a), b), c), d) and e).

Table 5. Indicators in the AWI of the "Environment" category, the definitions used for rating and their maximum individual score.

	Environment						
Score	**a) Natural light**	**b) Artificial light**	**c) Side openings**	**d) Draughts**	**e) Condensation**	**f) Noise**	**g) Grazing Time (days per year)**
3							>270
2.5							>230
2	Open fronted						>180
1.5	Very light	Very light					>120
1	Light	Light		None		No noise	>50
0.5	Medium	Medium	Yes	Sometimes	Good	Moderate	
0	Dark	Dark	No	Often	Ok	Noisy	
-0.5	Very dark	Very dark		Always	Bad	Intense	

.a) Open fronted housings are considered as optimal conditions for light. The percentage of windows area with light directed to the animals compared to the total floor surface is measured. Very dark: 0% (no natural light), very light 15%. b) If no artificial light was present, the score assigned was -0.5. During winter there is not much light during the day, especially going up the north, and farmers often have to work during the dark. No light when working in darkness is stressful for the animals and can be harmful for them and the stockperson. In case of neons or CFLs if less than one light per $5m^2$ was present the score assigned was 0. If one to 1.5 light per $5m^2$ were present the score was 0.5; 1 was assigned if between 1.6 to 2 lights per m^2 were present and 1.5 was assigned if more than 2 lights per $5m^2$ were present. In case of halogen lights 0 was assigned of there was less than one lamp per $15m^2$, 0.5 if there was between 1 and 1.5 lamp per 15 m^2, 1 if there was between 1.6 to 2 lamps per 15 m^2 and 1.5 if there was more than 2 lamps per m^2 d) Draughts were considered when air flow was greater than 0.2m/s. e) Air humidity was assessed subjectively with the forearms: if no humidity was felt the maximum score was assigned if humidity could be clearly felt the minimum score was assigned. f) Noise of the fans and ventilation systems were assessed subjectively, the maximum score was assigned if no ventilation system was present. If a ventilation system was present and the noise started to be irritating for the ear the minimum score was assigned. The total environment score equals to the sum of columns a), b), c), d), e), f) and g).

Table 6 **Indicators in the AWI of the "stockpersonship" category, the definitions used for rating and their maximum individual score.**

	Stockpersonship								
Score	**a) Trough cleanliness**	**b) Outdoor trough cleanliness**	**c) Feed cleanliness**	**d) Equipment**	**e) Cleanliness of animals**	**f) Lameness**	**g) Diseases**	**h) Background**	**I)Interest**
1	Clean	Clean	Clean	Good			None	Family	High interest
0.5	Medium	Medium	Medium	Medium	Clean	<5%	Few mild		Average interest
0	Insufficient	Insufficient	Insufficient	Defects	Medium	5 to 10%	Few severe	Other	Low interest
-0.5	Soiled	Soiled	Soiled	Bad	Soiled	>10%	Many severe		Not interested

a), b) and c) The troughs were considered clean when the water was clear, no algae could be seen in the water and no mud/slurry was present on them. Troughs were considered medium when the water was clear but small amount of algae could be found and/or few spots of mud/slurry of less than 2 cm of diameters were present on them. They were considered insufficient if the water started to be blurred but it was still possible to see through it, if the amount of algae was preponderant and if many spots of less than 2cm of diameter of mud/slurry were present on them. They were considered soiled if the water was blurred and it was not possible to see through it, if algae colonized the troughs and if mud/slurry covered the troughs or many spots of more than 2 cm of diameter were found. e) Clean animals were covered with less than 10% of slurry/mud, medium between 11 and 20% and soiled over 20%. g) The list of diseases and symptoms consisted of: respiratory diseases, scours, worms, parasites (mild diseases), BVD, BRD, Johne's disease, tuberculosis, leptospirosis and black leg (severe diseases). A maximum score of 1 was assigned if no diseases were reported. The score was 0.5 if up to 2 mild diseases or symptoms were reported. A score of 0 was assigned if the presence of one or two severe diseases or more than two mild diseases reported. A negative score of -0.5 was assigned if the presence of more than 2 severe diseases was reported or 4 mild diseases were reported. i) This indicator is subjective and the interest of the farmer is assessed with a five question questionnaire and a small interview. The total stockpersonship score equals to the sum of columns a), b), c), d), e), f), g), h) and i).

Table 7 **Active and illustrative variables used to run the PCA showing minimum and maximum scores.**

Active variables	Minimum score	Maximum score
Space allowance	0	2
Injurious protrusions	-0.5	0
Ease of locomotion	0	1
Grazing time	2.5	2.5
Age/group mixing	-0.5	1.5
Calving method	-0.5	1
Weaning method	-0.5	2
Type of floor	-0.5	2.5
Cleanliness of floor	0	1
Cleanliness of yard	0	1
Grassland	0.5	1
Natural light	0.5	2
Artificial light	-0.5	1.5
Side openings	0	1
Draughts	-0.5	1
Condensation	0.5	1
Noise	0.5	1
Water cleanliness	0	1
Water outdoors cleanliness	0.5	1
Feed cleanliness	0.5	1
Equipment	-0.5	1
Cleanliness of animals	-0.5	1
Lameness	-0.5	1
Health	-0.5	1
Background	0	1
Interest of the stockperson	0	1
Illustrative variables		
Number of animals	15	1000
Total score	17	36.5

[1] Higher scores represent less lameness.

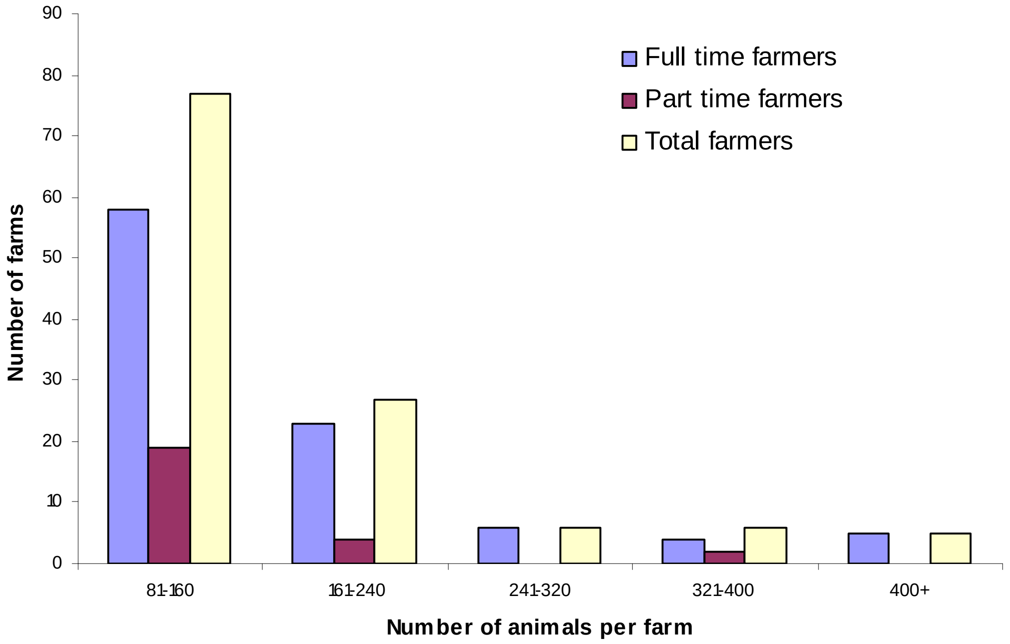

Figure 1 **Distribution of the number of total cattle on farms. General mean = 131 animals per farm. First quartile corresponds to 59 animals, median to 100 animals and the third quartile to 150 animals. FT = full time farmers, PT = part time farmers, TOT = FT+PT.**

Table 8 **Number of full (FT) and part time (PT) farmers, respective mean of the number of animals owned and mean scores of the AWI and each category.**

	FT	PT	P-values
N	125	69	
Mean number of animals	160 ± 13.5	80 ± 7.7	P<0.001
AWI	65 ± 7%	65 ± 7%	NS
Locomotion category score	54 ± 12%	55 ± 12%	NS
Social; interactions category score	48 ± 12%	52 ± 11%	P=0.001
Environment category score	88 ± 7%	87 ± 7%	NS
Flooring category score	50 ± 15%	48 ± 12%	NS
Stockpersonship category score	88 ± 9%	86 ± 9%	NS

NS = non significant.

Table 9 Significant differences found in the individual indicators of the AWI.

Category	Indicator	Sign.	ranks
Social	Grouping	P<0.001	PT >FT
	Weaning	P = 0.03	PT>FT
Stockpersonship	Cleanliness of animals	P = 0.03	FT>PT
	Lameness	P = 0.01	FT>PT
	Interest	P = 0.05	FT>PT

FT = full time farmers, PT = part time farmers.

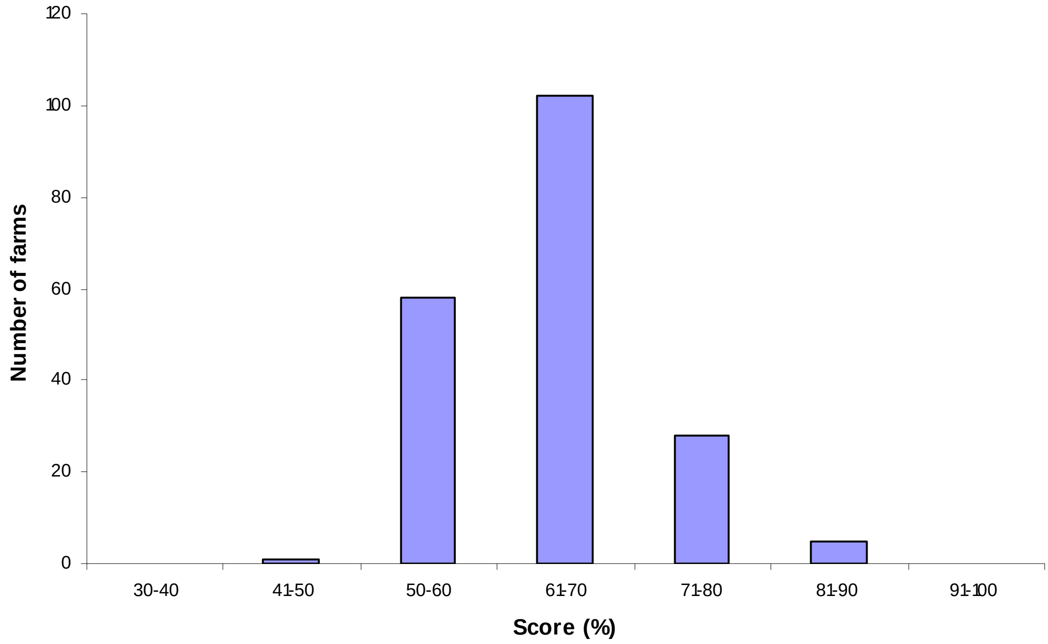

Figure 2 **Distribution of the AWI shown in percentage of the maximum score possible (normally distributed). The AWI ranged from 54% and 83% with a mean of 65% (s.d. = 6%) of the maximum score.**

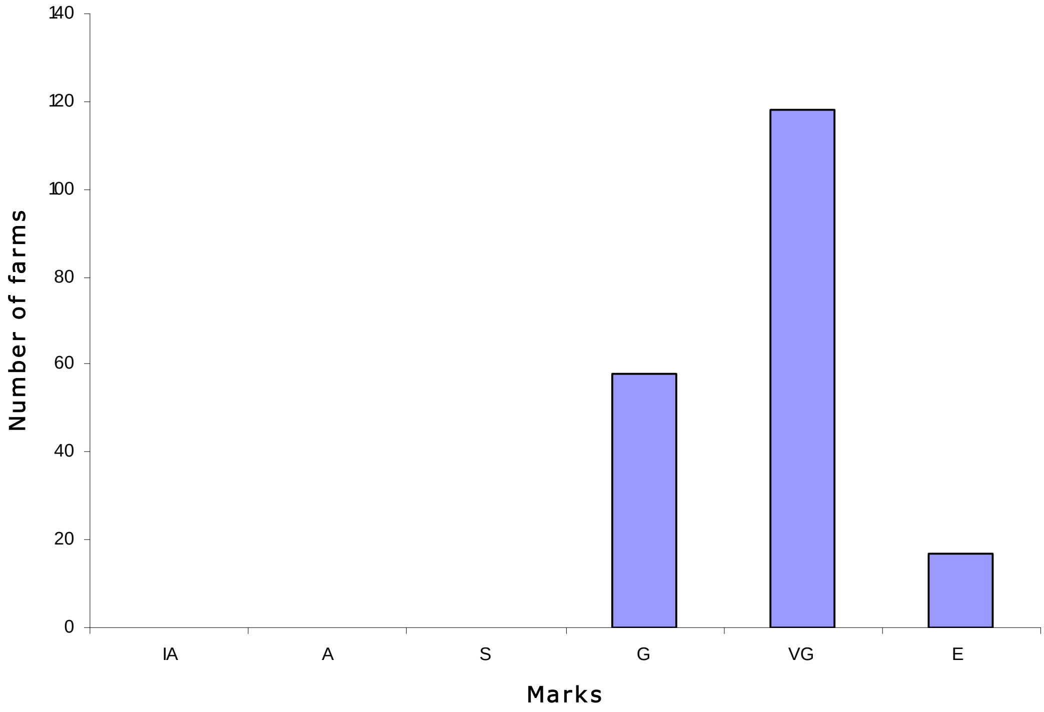

Figure 3 **Distribution of the welfare ranks of the farms. IA = inadequate; A = adequate, S = satisfactory (1 farm); G = good (58 farms), VG = very good (118 farms); E = excellent (17 farms).**

Table 10 **Correlations that were significant between the total number of animals on-farm and the AWI, the category scores and the individual indicators scores.**

Indicators	**Significance**	R_s
Number of acres	P = 0.001	0.17
Calving	P = 0.04	-0.13
Type of floor	P = 0.04	-0.13
Locomotion score	P = 0.03	-0.14
Noise	P = 0.03	-0.14
Natural light	P = 0.02	-0.15
Space allowance per animal	P = 0.007	-0.19
AWI	P = 0.001	-0.21
Weaning	P < 0.001	-0.23
Grouping	P < 0.001	-0.32
Social interactions score	P < 0.001	-0.35
Health	P < 0.001	-0.8

Table 11 **Correlations that were significant between the interest of the stockperson and the AWI, the category scores and the individual indicators scores.**

Indicators	Significance	R_s
Stockpersonship score	P < 0.001	0.67
Feed cleanliness	P = 0.012	0.62
Cleanliness of floor	P = 0.01	0.47
Outdoors water cleanliness	P = 0.001	0.44
Lameness (less)	P < 0.001	0.43
AWI	P < 0.001	0.42
Health	P = 0.023	0.42
Cleanliness of animals	P = 0.003	0.40
Artificial light	P = 0.025	0.37
Weaning	P = 0.023	0.35
Space allowance per animal	P = 0.016	0.33
Environment Score	P = 0.009	0.30
Locomotion score	P = 0.023	0.29
Social score	P = 0.026	0.27

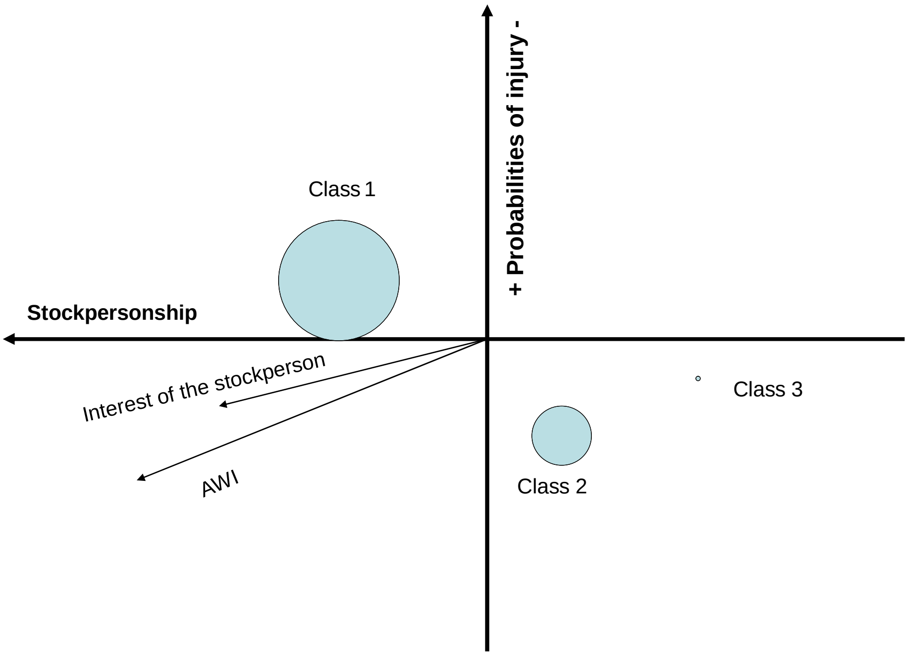

Figure 4 **Representation in the principal plan of the PCA of the 3 classes obtained with the hierarchical classification. The first factor, "stockpersonship" represented 11% of the variance. The second factor, "health and probabilities of injury", represented 9% of the variance. Class 1 (mean AWI = 66%, higher interest of the farmer) N = 130; Class 2 (mean AWI = 62%, lower interest of the farmer) N = 59; Class 3 (mean AWI = 54%, lowest interest of the farmer) N = 5.**

3.0. Investigation and specificity of behavioural fear responses of heifers to different fear-eliciting situations involving humans.

3.1 Introduction

It is well recognised that fearfulness is a major component that influences the welfare for both animals and stockpersons (Waiblinger *et al*., 2006). The importance of fearfulness on an animals' welfare is amplified by the fact that farm animals show a strong attraction to humans, whether by curiosity or because they usually receive their feed from humans (positive experience). However, husbandry management procedures, for example, castration, dehorning and changes in their social and physical environment may induce fear responses in animals (Boissy, 1995; Hemsworth and Coleman, 1998). Contact between cattle and stockpersons has decreased, with increased mechanisation, size of herds. In some countries, like Ireland, with the increased involvement of farmers in part-time farming, resulting in less time allocated to animals by the stockperson such that animals may not have the same exposure to handling by humans (Rushen *et al*., 1999a) and interactions could be limited to negative ones (change of environment (novelty) or administering vaccine for example). A poor human-animal relationship (HAR) leads to reduced animal welfare which impacts on productivity, quality and profitability of farm animals (Hemsworth and Coleman, 1998; Seabrook, 1972; Rushen *et al*., 1999a). The HAR could be assessed from the stockpersons' responses to a series of statements in a questionnaire (Hemsworth and Coleman, 1998) or by observation of the stockperson during his/her daily routine but these methods can be easily manipulated.

Alternatively the reactions of animals to their caretaker or unfamiliar people through fear and avoidance (Hemsworth *et al.*, 2002) or ease of handling (Boivin *et al.,* 1992b) can be measured. Fear is an emotion and thus by definition is punctual, whereas the fact of being fearful depends on the personality of the animal. Generally, fearfulness is assessed using behavioural tests that are grouped in three categories: stationary human, moving human and restraint/handling (Waiblinger *et al.*, 2006). All these tests assess fearfulness of the animals in different situations, such as isolation, presence of a novel object, a novel environment or presence of a stationary or moving human. In a given situation, animals may perceive an interaction as positive, neutral or negative. There is still a debate about fearfulness. The fear of human is seen for some as a personality trait (Boissy and Bouissou, 1988; Lansade and Bouissou, 2008), thus sTable over time or space, and independent of the conditions (Costa and McCrae, 1986). On the contrary (Boivin *et al*., 1994) it is seen as the expression of the HAR that has developed over time. Furthermore, fear could have several dimensions (Miller *et al.*, 2005; Petherick *et al.*, 2009), namely, …..

It is still debated that the assessment of fear of humans could just be the assessment of a general fearfulness. Some studies showed that it is the result of the relationship between the animals and the humans (Hemsworth *et al*., 1996, 2000) whereas others showed it is a biological trait (Boissy *et al.,* 2005). Jones (1996) reported that the perception of humans by animals and their responses to some stimuli was influenced by their personality traits, such as fearfulness. Many tests have been used and are designed to assess fearfulness. Fearful animals that have defensive reactions are more difficult to handle (Boivin *et al*., 1992). Kilgour *et al*. (2006) found that a restraint test (also called docility test) and tests assessing the fear of human (flight test, open-field, etc.) were the best to assess the individual fear responses of cattle. Hemsworth and Coleman (1998) reported that potentially, the most frightening event for farm animals is their exposure to humans and changes within their social or environmental conditions. Consequently, the HAR is a potential indicator of animal welfare. However, it is necessary to examine if the responses measured in different tests, supposed to measure fearfulness, are actually valid in the dimension they are measuring. If two variables measured are in the same dimension and thus measure the same thing, they should be correlated (convergent validity). On the contrary, if they are measuring two different dimensions, they will be uncorrelated or specific (divergent validity). Most studies investigating fear responses in cattle have focused on dairy cows (reviewed by Burrow, 1997). There is a lack of such information on beef cattle and more specifically, a deficiency regarding the specificity of fear responses in cattle. In this study, the fear responses (according to the literature) of heifers towards humans were measured using different tests where animals were appraised under different conditions. Thus, if fearfulness is a personality trait and thus general, the measures collected would be correlated, whereas if fearfulness was specific it would result in uncorrelated measures.

The objectives of this study were: (i) to investigate the reaction of beef heifers to different stress-test conditions, and (ii) to test the specificity of the fear variables. To achieve those objectives, four behavioural tests - flight, docility, fear and chute tests - were performed.

3.2 Material and methods

Animals

The study was conducted at Teagasc, Grange Beef Research Centre (Co. Meath, Ireland). Weanling heifers (7-12 months of age), comprising 44 purebred Simmental (PB) and 22 Simmental × Friesian-Holstein (CB) were purchased off-farm and assembled at the research centre. Upon arrival they were turned out to pasture where they spent a minimum of one month grazing. At the end of this period the heifers were assigned to pen groups (5 or 6 animals per pen) in a slatted floor shed with a Calan gate feeding system. Heifers were weighed and blood sampled every 21 days (day 21, 42, 63 and 84 post-housing) following a 3-week adaptation period to the diet and Calan gate system. Initial live weight was 328 ± 40 kg for the PB and 275 ± 57 kg for the CB.

All heifers were vaccinated against BVD, RSV, PI-3, IBR, Blackleg and treated for gastro-intestinal worms and ecto-parasites upon or after arrival to the research centre. Post-housing, animals were treated for fluke and, ecto-parasites as deemed necessary.

Tests

The flight, docility and fear tests (described below) were carried out over three consecutive days, in that order, commencing on day 30 and again on day 80 post-housing. The chute test was performed on day 84 post-housing.

Flight test

The flight test assessed the latency time to join a group of animals and the avoidance distance (minimum approach distance by a human before an animal fled). The test was performed in an unfamiliar alley (5 x 40 m) that was constructed adjacent to the housing facility (Figure 1). The alley had clean floors and solid walls that were marked in metre lengths, to permit measurement of distance. At one end of the alley, separated by a gate, a pen (5 x 5 m) was constructed to hold seven heifers ("non-test peers"). At the other end of the alley a holding area was constructed, in which each pen of heifers was placed prior to individual animal testing. The heifers from one pen were randomly taken from their home pen to the holding area with gentle and calm movement one hour after feeding. One heifer of the group was randomly separated from the others and allowed to enter the alley. The latency time in seconds to join the non-test peers group was measured (LAT1 refers to the first test session and LAT2 to the second test session). When the tested heifer arrived at the non-test peers' pen or after 30 seconds if the heifer did not join the peers' pen, the operator (unfamiliar human) entered the pen and walked slowly (1m/s) towards the heifer. The operator stopped when the heifer moved two steps after having shown an alert posture (low head, glance towards the operator and raised ears). The distance in metres between the heifer and the operator was then measured (DIST1 for the first test session; d 30: DIST2 for the second test session; d 81). The latency time and the minimum approach distance values were used to assign scores (Table 1). When all heifers within a group were tested, the group was returned to its home pen and another group was led to the holding area.

Docility test

The docility test was adapted from Le Grignard *et al.* (2001) and was designed to measure the passivity of animals. A 5 x 5 m pen with solid walls, to ensure that tested heifers did not have visual contact with their peers, was used for the test and a digital camera was positioned above the pen to record the behaviour of the animals during the test. A 1.5 x 1.5m square was drawn on the floor at the opposite corner of the pen, from the entrance gate (Figure 2). The animals were fed one hour prior to the test. The animals were gently brought from their home pen to a holding area that was in front of the test pen and remained there for 10 minutes before the test started. One animal was taken randomly from the group and gently brought to the test pen. The animal was left alone for 30 seconds before an unfamiliar human entered the pen and tried to encourage the heifer to move into the marked square and then attempted to contain the heifer within that square for 30 seconds using slow arm movements, calm voice and a stick. The test was terminated if the heifer couldn't be moved to the corner within 1 minute, if she threatened or charged the operator, if she came out of the corner or if she could be contained in the corner for 30 consecutive seconds. The time taken to enter the square area (TC1 for the first session; d 31; TC2 for the second session; d 82) and the time the heifers could be contained in the square (TR1 for the first session; d 31; TR2 for the second session; d 82) was recorded. The animals were assigned scores ranging from 6 to 0 depending of their docility or aggressiveness, with a score of 6 being the most docile animals and a score of 0 was given if the heifer threatened the operator (Table 2).

Fear test- reaction to humans

The fear test is an open-field test which measured the fear reactions of the animals to different conditions. The test was adapted from Mazurek *et al*. (2007). The animals were fed one hour prior to the test. An unfamiliar human conducted the test. The heifers were brought from their home pen to a holding area for 15 minutes. After that they were gently moved to a pen, adjacent to the test pen. The two pens were separated by a tubular gate (transparent) and a sliding solid wooden partition. The test pen was 9 x 4.5 m with 1.5 m side squares drawn on the floor and two digital cameras were positioned overhead to record the behaviour of the animals during the test. The squares were given different values depending on their distance from the stimulus (Figure 3). Scores were awarded as follows: 0 for the square where the stimuli was located, 1 for the adjacent squares, then 2, 3, 4 and 5 for the furthest squares (Figure 3). One heifer was randomly taken from the group to the test pen and was left alone for 60 s (phase 1). The operator entered the pen and placed a bucket containing concentrates on the ground, and exited (phase 2). After 30 s, the operator entered the pen and positioned themselves 10 cm behind the bucket from the bucket containing concentrates for 30 s (phase 3). After 30 s, the sliding wooden partition was opened and the heifer was allowed to see her peers in the adjacent pen for 30 s (phase 4) (Figure 3). The recorded parameters were the number of squares crossed (NBS) during phase 1 (NBS11 for the first session; NBS12 for the second session), 2 (NBS21 for the first session, NBS22 for the second session), 3 (NBS31 for the first session; NBS32 for the second session) and 4 (NBS41 for the first session, NBS42 for the second session); time eating (TE21, TE31 and TE41 for the respective phases of the first session; TE22, TE32 and TE42 for the respective phases of the second session), time spent at less than 1 m from the stimulus (AP21, 31 and 41 for the respective phases of the first session; AP22, AP32 and AP42 for the respective phases of the second session), the mean distance between the heifer and the stimulus (MD21, MD31 and MD41 for the respective phases of the first session; MD22, MD32, MD42 for the respective phases of the second session; the latency to interact (sniffing or licking) with human (LTH) during phases 3 (LTH31 for the first session; LTH32 for the second session) and 4 (LTH41 for the first session; LTH42 for the second session). The number of squares crossed during phase 1 was halved in order to have the number of squares crossed of a 30 s period. A square was considered crossed when the animal placed its head and two front feet in it. The mean distance between the heifers and the stimuli was calculated as below:

$$MD = \frac{U*0+V*1+W*2+X*3+Y*4+Z*5+1}{U+V+W+X+Y+Z+1}$$

The values collected were used to assign scores from 6 or 5 to 0, depending on the variables, as shown in Table 3. The quietest animals were assigned a score of 6 whilst the most fearful/ aggressive ones were assigned a score of 0.

Chute test.
The chute test assessed the reactivity of animals before, during and after blood sampling. A digital camera was positioned over the race and chute. Three operators assisted with the test, one to bring the animals to the race, one to handle the heifers' head and one to perform the blood sampling. The heifers were removed from their home pen to a holding area and were not mixed with unfamiliar animals. The animals were weighed first, and then moved to a holding pen (Figure 4). The chute gate was opened and one heifer was randomly led into the race by the first operator. The heifer was restrained with a head catching gate for blood sampling. Once restrained in the catching gate, the animal was left alone for 5 s. After the 5 s had elapsed, the head of the heifer was restrained by hand to permit blood sampling via jugular venipuncture by the third operator. If the animal was too difficult to handle, a nose tongs was used. A jugular clamp was used to assist with blood sampling. On completion of the test, the heifer was released to a post-test holding pen (Fig. 4).

To standardise the test procedure it was demonstrated, using pilot animals, to the personnel on the day before. Data recorded were, willingness of the animal to enter the race; head, feet and tail movements once restrained at the catching gate; difficulty to manually handle during blood sampling; exit speed (time spent to move 1.7 m) of the animal after blood sampling (Table 4). An "agitation" variable was calculated by summing up the head, feet and tail movements at the catching gate. Scores were assigned from 1 to 4 or 1 to 3 depending of the variable (Table 5).

Operators
The same (unfamiliar) operator performed the two test sessions of the flight test and of the fear test. Another (unfamiliar) operator performed the two test sessions of the docility test. Three other operators (unfamiliar) performed the chute test. The order of the tests was flight test (operator 1), docility test (operator 2), fear test (operator 1) and chute test (different operators). All operators wore identical clothes (clean blue overall suit).

Statistical analysis
Data were checked for normality using the Shapiro-Wilk test. Data that was not normally distributed was analysed using non parametric tests. Breed type effects were tested prior to analysis with a Mann-Witney *U* test. Intra-test correlations and inter-session correlations among one test were checked with a Spearman's ranks correlations test. Differences in behaviour between the first and the second session of each test were analysed using the Wilcoxon matched-pairs test. The differences in behaviour during each phase of the fear test were analysed with the REML procedure for repeated measurements (Genstat 11th edition, VSD, UK). The means of the two sessions for each variable of the flight, docility and fear tests were calculated and used together with the variables from the chute test to perform a Principal Component Analysis (Copri procedure) with DECISIA SPAD 6.5.

3.3 Results

Breed type effect
An effect of breed type (Mann-Whitney *U* test: *U* = 258, N = 66, P < 0.001) was found for the avoidance distance during the second session of the approach test (DIST2). Purebred animals had a greater (P<0.05) mean distance of 14.6 ± 1.0 m, whereas the CB had a mean distance of 7.0 ± 0.9 m. No effect of group was found among the other variables (P > 0.05), thus the statistical analysis was performed independently of breed type (with the exception of the avoidance distance of the second session of the flight test).

Flight test
The mean latency time in seconds (s) to join the peers was 15.2 ± 0.2 s, (N = 66) for the first session (LAT 1) and 15.9 ± 0.9 s for the second session (LAT2) (N=66) with no difference (P > 0.05) or correlation (P>0.05) found between the two sessions.

The mean distance that the heifers allowed a human to approach them was 9.0 ± 0.5 m during the first session (DIST1). During the second session (DIST2) the PB had a mean distance of 14.6 ± 1.0 m, whereas the CB had a mean distance of 7.0 ± 0.9 m. The minimum avoidance distance did not differ (P>0.05) between the sessions for the CB, whereas in the PB it was longer (P < 0.001) during the second test session. No significant correlation was found between DIST1 and DIST2 for each breed type (PB: N = 48, P = 0.68; CB: N = 18, P = 0.64).

The latency to join peers during the first session of the flight test (LAT1) was correlated (r_s = 0.49, P < 0.001) with DIST1, whereas LAT2 was not correlated with DIST2 for the PB (P = 0.23) or the CB (P = 0.11). During the first test session, one animal scored 1 for LAT1, 12 animals scored 2, 28 animals scored 3, 11 animals scored 4, five animals scored 5 and 8 animals scored 6. During the second test session zero animal scored 1 for LAT2, 20 animals scored 2, 19 animals scored 3, eight animals scored 4, six animals scored 5 and 13 animals scored 6 (Figure 5). Regarding the avoidance distance, during the first test session one animal scored 1, five animals scored 2, 26 animals scored 3, 24 animals scored 4 and 23 animals scored 5. During the second test session, 11 animals scored 1, 10 animals scored 2, 25 animals scored 3, 15 animals scored 4 and 18 animals scored 5 (Figure 6).

Docility test
During the first test session, two PB animals threatened the operator. Two other PB and three CB could not be moved to the corner of the test arena within one minute. During the second test session, two PB threatened the operator (the same animals as during the first session). Two other PB and three CB could not be moved to the corner. No difference (P > 0.05) in scores was found between the two test sessions for the time required to move the animals to the corner (TC) and the time they could be contained in the corner (TR). The distribution of scores is shown in Figures 7 and 8. Correlations were found between TR1 and TR2 (r_s = 0.36, P = 0.008); between TC1 and TR1 (r_s = 0.33, P = 0.001) and between TC2 and TR2 (r_s = 0.62, P<0.001).

Fear test
During the first session, heifers crossed a greater number of squares (P < 0.05) during phase 1 than during phases 2, 3 and 4, which did not differ (P > 0.05). There was no difference (P > 0.05) in scores for the number of squares crossed between the phases during the second session of the test (Table 6).

The number of boxes crossed during phase 1 did not differ (P > 0.05) between the two sessions, whereas there were differences between the sessions (animals more fearful the second time) for phases 2 (P < 0.001), 3 (P = 0.04) and 4 (P = 0.001). Correlations were found for the number of boxes crossed during phase 3 (r_s = 0.44, P=0.002) and phase 4 (r_s = 0.68, P < 0.001) between the two sessions (Table 7).

Heifers showed significant differences between the scores of the mean distance from the stimulus during the first session. Mean scores of phase 3 were lower (P < 0.001) than scores from phases 2 and 4, and mean scores of phase 2 were lower (P=0.01) than the scores of phase 4. During the second session, no differences (P > 0.05) were found between mean scores of phases 2 and 3. Phase 4 was higher in mean score than phases 2 (P < 0.001) and 3 (P < 0.001) (Table 6).

Significant differences in scores were found for phase 2 (P < 0.001) and 4 (P = 0.04) between the two sessions, whereby animals stayed further away the second time. Correlations were found between the two sessions for the mean distance from stimulus during phase 3 (r_s = 0.34, P = 0.03) and 4 (rs = 0.41, P = 0.02).

The heifers spent more time eating during the second phase compared to the third (P < 0.001) and fourth (P < 0.001) phases of the first session. No difference (P > 0.05) was found between the scores of phase 3 and 4. During the second session they spent less time eating during phase 4 than the other phases (P < 0.001).

Significant differences were found during phases 2 (P < 0.001), 3 (P = 0.04) and 4 (P < 0.001) between the two sessions, with animals eating less the second time. Correlations were found for the time eating between the two session for phase 2 (r_s = 0.47, P = 0.004), 3 (r_s = 0.79, P = 0.007) and 4 (r_s = 0.79, P = 0.001).

Differences in scores were found between phase 4 and phases 3 (P < 0.001) and 2 (P = 0.001) in the time spent at less than 1 square (1.5 m) from the stimulus during the first session.

There was also a difference between phases 2 and 3 (P < 0.001). During the second session, phase 4 differed (P<0.001) from phases 3 and 2.
Significant differences in scores were found between the two sessions for phases 2 (P<0.001) and 4 (P = 0.001), the animals being more fearful the second time. There was a correlation (r_s = 0.57, P < 0.001) for the scores of phase 4 between the two sessions.

Significant correlations (P<0.05) were found between the two sessions for the number of squares crossed during phases 3 and 4, the mean distance from stimulus for phases 3, and 4, the time eating during every phase, the latency time to interact with a human for phase 4, and the time spent at less than 1.5m from the stimulus for phase 4 (Table 7).

Chute test
Sixty-two percent (41 animals) of the animals entered the head gate willingly, 22.8% (15 animals) halted before entering the head gate and 15.2% (10 animals) had to be forced to enter the head gate; 9.1% (6 animals) were easy to handle during blood sampling, 21.2% (14 animals) reacted negatively to the procedure, 68.2% (45 animals) were difficult to blood sample and 1.5% (1 animal) could not be blood sampled without restraining the animal with a nose tongs. For most heifers overall agitation was low: 54.5% (36 animals), 25.8% (17 animals), 12.1% (8 animals) and 7.6% (5 animals) had respective scores of 4, 3, 2 and 1. Corresponding percentages for speed of exit from the chute after blood sampling were: 15.2% (10 animals), 34.8% (23 animals), 47.0% (31 animals) and 3% (2 animals) (Figure 9).

There was a correlation between the overall agitation scores (AGIT) and the easiness to enter the chute score (ENT) (r_s = 0.40, P=0.01) and the easiness to handle during blood sampling (RES) (r_s = 0.45, P = 0.006).

Intra and inter-tests correlations
The time spent at less than one box from the stimulus during the fourth phase of the fear test (AP4) was correlated with 18 variables out of 22. The number of squares crossed during phases 3 (NB3) and 4 (NB4) were correlated with 17 out of 22. The avoidance distance during the flight test (DIST), the time needed to move the animals to the corner during the docility test (TC), the number of squares crossed during the first phase of the fear test (NB1),the time animals have been eating during phase 2 (TE2), the mean distance between the animals and the stimulus, the time animals ate and the time spent at less than one box from the stimulus during phase 3 (respectively MD3, TE3 and AP3), the mean distance between the animals and the stimulus and the time they ate during phase 4 (respectively MD4 and TE4) were correlated with 16 other variables. The number of squares crossed and the time spent at less than one box from the stimulus during phase 2 (NB2 and AP2) were correlated with 14 other variables. The mean distance between the animals and the stimulus during phase 2 (MD2) was correlated with 13 other variables. The time the animals could be contained in the corner during the docility test (TR), the latency time to interact with the human during phases 3 and 4 of the fear test (LTH3 and LTH 4) were correlated with 12 variables. The overall agitation during the crush test was correlated with 4 other variables, the speed of exit (SPE) was correlated with 2 other variables the willingness for the animals to enter the race (ENT) and the easiness to blood sample them (RES) were correlated to only one other variable. The r values ranged from 0.24 to 0.97 (Table 8).

The PCA showed that the first component represented 37% of the variability and was described by the variables of the docility test and the variables measuring the number of squares crossed (NB) and the distance between the animals and the stimulus (MD and AP) of phases 3 and 4 of the fear test. The second factor represented 12% of the variation and was described by the agitation (AGIT) during the chute test. Thus, the two first synthetic factors explained 49% of the variability. The third factor was described by the latency time to join the peers during the flight test and the speed of exit from the chute (7%).

3.4 Discussion

The present study showed that not all collected measurements were correlated, consequently animal responses were condition specific and that fear of humans would differ from the general reactivity of the animals. The presence of conspecifics calmed the animals as found by Grignard *et al.* (2001) and reduced the distance between themselves and the human indicating that the fear of social isolation was greater than the fear of a human or that the motivation to join the peers was more important to the motivation of avoiding the human. The motivation to stay close to their peers was thus more important than their fear of a human. The animals showed more accentuated responses between the two test sessions of the fear test and for the avoidance distance during the flight test. The differences observed in the avoidance distance between the two test sessions of the flight test could be due to environmental factors. All the tests were performed indoors except the flight test, thus environmental changes could have occurred during the test and impacted on the animals' behaviour. This could explain the differences between the two breed types. Numerous studies showed an effect of breed on behaviour (Boivin *et al*. 1992, 1994; Grignard *et al*, 2001; Boissy *et al*., 2005). Grignard *et al*. (2001) showed an effect of genetic origin in the docility of animals. Boissy *et al*. (2005) reported that genetic factors underlie fear responsiveness. It was also reported that the social environment and the previous experiences could change the behaviour of animals (Boivin *et al*., 1992, 1996, 2001; Krohn, 2001; Lensink, 2001). In this study, the CB heifers were artificially (bucket) reared whereas the PB heifers were suckled on their dam. In addition to the different rearing conditions, they were sourced from several farms so it was not possible to conclude about the differences observed in the avoidance distance. Nevertheless no other difference was evident in the measured variables, which suggests that the behavioural responses would be more related to the animals' history than to genetic factors.

Between the different phases of the fear test and between the two test sessions of this latter, differences in fear responses for the majority of the variables could be observed, but the not during the docility test. This could be due to the accuracy of the tests as the docility test measured only two variables, whereas the fear test measured up to six variables in four different conditions. The animals showed more intense fear reactions during the second test session of the fear test for phases two and four. The scores of phase three were not different between the two test sessions but this may be explained by the fact that the scores were already low for this phase during the first test session and it would have been harder to have a lower score. Nevertheless, the lower scores that were observed during the second test session of phase four show that the fear responses have increased between the two test sessions.

Grignard *et al*. (2001) found that the behavioural reactions of animals during a docility test were correlated to the reactions during the chute test. In the present study, the overall agitation in the chute test was the only variable that was correlated with the variables of the docility test, however, the correlations found were low. As reported by Grignard *et al.* (2001) the positive correlations found during the chute tests gave negative correlations during the docility test. The absence of a correlation between the willingness to enter the race and the ease of restraining with other variables could be due to the fact that the chute test was performed only once.

The animals reacted consistently for 63% (12 out 19) of the variables measured in all tests between the two test sessions. The consistency was the lowest when the animals were isolated or in the presence of a stationary human and the fear responses were more consistent when the tested animals could see there peers. Grignard *et al.* (2001) proposed that there was a general reactivity of the animals to handling whether they are restrained or not. In the present study, similar results were found for the agitation of the animals during the chute test but the animals reacted independently for ENT and RES. Besides, the agitation of the animals during the chute test was only correlated (low correlation) to the variables of the docility test but not to the variables of the other tests. Thus, the variables assessing fear in restrained and unrestrained conditions seem to be diverging. The PCA showed that the fear responses could be regrouped in three synthetic factors. The two first factors were described by the fear responses measuring the agitation (number of squares crossed, agitation during blood sampling), avoidance of a human and docility of the animals. These results are similar to Kilgour et al. (2006). The willingness to enter the chute and difficulty to restrain them showed the motivations of the animals to avoid the stimuli, were correlated with the agitation level and could therefore indicate fear. The correlation between the agitation and the difficulty to restrain the animals was low because only strong head swings could make the procedure harder and the animals' head was partially blocked in the catching gate, thus the agitation score resulted more of feet and tail movements that did not hamper the procedure. The speed of exit from the head gate was correlated to the latency time to join the peers during the flight test but these two variables were not correlated to any other variable. This could mean that these variables may be measuring another characteristic unrelated to fear such as the natural movement of the animals for example.

Fearfulness was identified as a composite trait in Japanese quails by Miller *et al*. (2006), who showed that the converging validity was good within the same test but lower between tests. On the contrary, Lansade & Bouissou (2008) reported that the behavioural fear responses across tests were consistent in horses. Boissy & Bouissou (1995) showed that "the propensity for an individual to react excessively to a given test was related to its reactivity to another frightening event" and Boissy *et al*. (2005) showed that usually the responses to high fear eliciting events were negatively correlated to the ones inducing low stress events. In agreement with the findings of Boissy *et al.*

(2005), in this study, the fear of a human when the animal was restrained in a chute was not correlated with the other types of fear with the exception of a low negative correlation between the scores of the docility test and the agitation score. The reason why the animals didn't react to the chute test consistently to their reactions to the other tests may be due to the fact that in unrestrained conditions the animals are free to roam and can avoid humans, whereas in restrained conditions they cannot flee and have to undergo the human handling. Thus, the human could be seen as a predator (Hemsworth *et al.*, 2001) and any animal would react excessively compared with the unrestrained conditions.

When entering a novel environment such as an open-field arena (the fear test in this study) animals showed higher fear levels than when the food was offered. The most frightening event was the presence of the stationary human when the animals couldn't see their peers. Generally, the presence of a human is a frightening stimulus (Rushen *et al*., 1999a; Mazurek *et al*., 2007). However, if the peers could be seen the animals reduced the distance between themselves and the human. Waiblinger (2003) suggested that the approach distance of an animal could result of a conflict of motivations between what is attractive to them (e.g. peers or food) and what they are afraid of (e.g. humans). Stankowich (2008) also found that ungulates pay attention to approacher behaviour and have greater perception of risk when disturbed in open areas. The time eating during the different phases of the fear test was correlated for each phase and between sessions suggesting that the motivation to eat (or gluttony) could be interacting with the fear responses. This was in agreement with Millet *et al*. (2006). It could also be that less dominant animals can withstand isolation from the group better than dominant individuals and take advantage of the situation to eat the concentrates (Mazurek *et al*., 2007).

The fear of the animals in different conditions seemed to evolve over time (flight and fear test) and sometimes not (docility test). Between the two test sessions, the animals were led to the chute and were blood sampled and weighed on two occasions. This indicates that two repeats of a negative experience were enough to change the reactions of animals. However, these results come from tests where the animals were isolated to limit the influence of the social environment and the fear reactions of the animals when they are in their group could be attenuated.

In conclusion, this study showed that fear of humans is not a unitary trait as reported by Petherick (2009). Most of the data showed a convergent validity of the different variables meant to assess fearfulness, however, in the situation where the heifers were restrained, the data were diverging from the others thus may be assessing another facet of fear or assess something not related to fear. The result of the PCA tended to confirm this and it seems that fear has different facets. Only a bit more than half of the reactions were consistent between the two test sessions. The fear levels of animals can change over time and the changes can be observed quickly. The different motivations of an animal can also affect their reaction to a frightening event. The most frightening events of the fear test were the social isolation and the presence of a stationary human. Tests measuring fear of animal or assessing the HAR should consider the different dimensions of fear to have a more accurate assessment.

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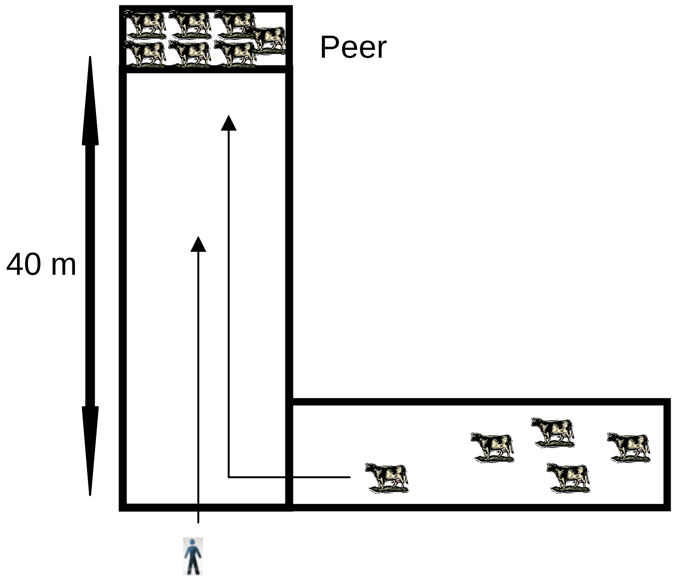

Figure 1. Scheme of the flight test pen. A marking was placed at 1 metre distance apart along the wall of the 40m alley. Seven heifers served as peers and were located in a pen at the end the alley to attract the tested animals.

Table 1. References used to assign scores to the measurements of the flight test variables.

Score	1	2	3	4	5	6
Latency time	0.1-5.0s	5.1-10.0s	10.1-15.0s	15.1-20.0s	20.1-25.0s	25.10s+
Avoidance distance	21m+	16-20m	11-15m	6-10m	1-5m	

The higher the score the better the animals responded to the situation.

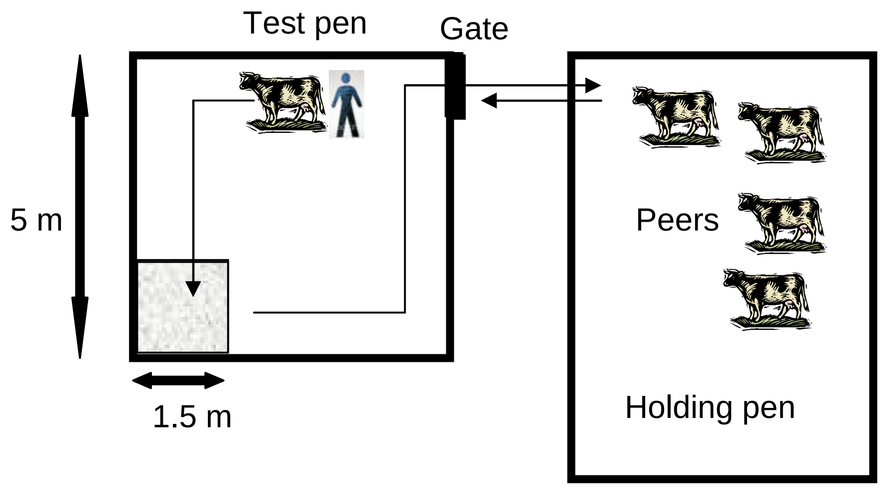

Figure. 2. Docility test procedure: a heifer entered the test pen and stayed alone for 30 seconds. The operator entered the pen after the 30seconds and tried to lead the animal to a 2.25m^2 square drawn on the floor (TC) of the test arena. Once the animal entered the corner, the operator tried to maintain it in the corner for 30 seconds (TR). If the animal was threatening the operator or if it couldn't be led to the corner after 1 minute, the test was terminated and a score of zero for TC and TR was assigned.

Table 2. References used to assign scores to the measurements of the docility test variables.

Score	0	1	2	3	4	5	6
TC	Threat	51+	41-50	31- 40	21-30	Nov-20	≤10
TR	Threat	0-5	6-10	11-15	16-20	21-25	26+

TC: time needed to lead the animal to the drawn square in the corner. TR: time the animal could be contained in the square in the test pen. Units in seconds.

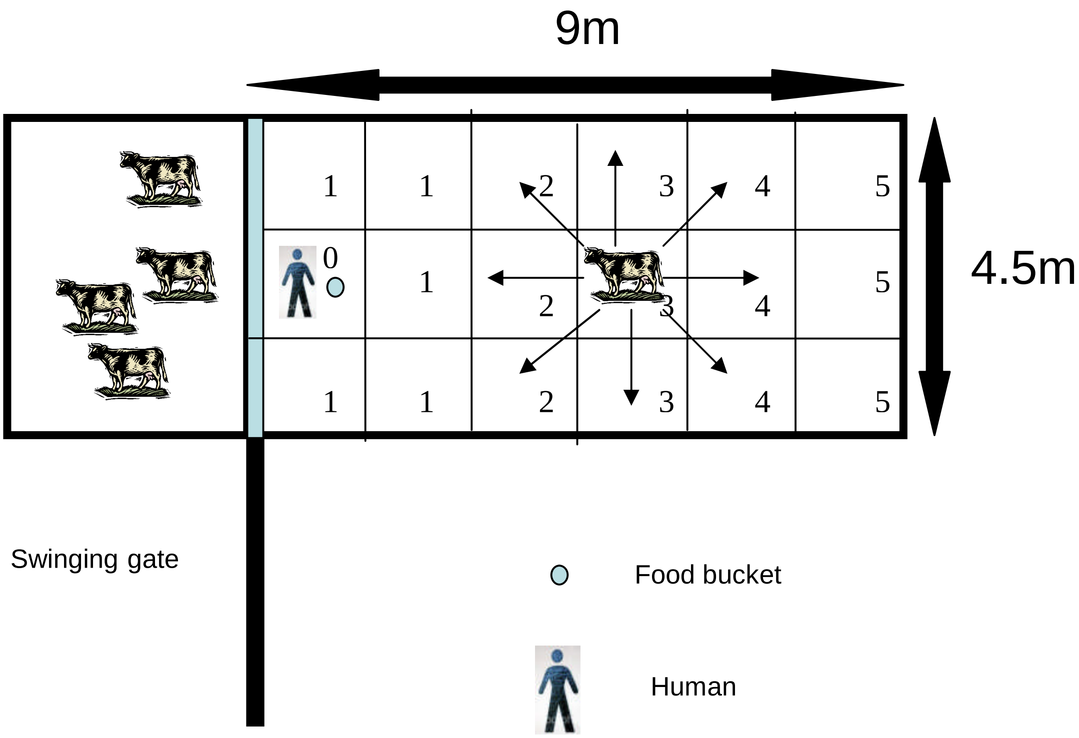

Figure 3. Fear test procedure: one heifer was taken at random to the test pen and remained there for 1 minute alone (phase 1). The experimenter entered the pen and placed a feed bucket containing concentrates in the pen, and exited (phase 2). After 30 s, the experimenter entered the pen and positioned themselves 10 cm from the feed bucket for 30s (phase 3). After 30s, a swinging gate was opened and the heifer was allowed to see its peers on the adjacent pen for 30 seconds (phase 4). The numbers in the squares represent the value given to the squares for the mean distance to stimulus calculation.

Table 3. References used to assign scores to the measurements of the fear test variables.

Scores	0	1	2	3	4	5	6
NBS	Threat	41+	31-40	21-30	11-20	01-10	
MD	Threat	3.1+	2.1-3	1.1-2	0.1-1	0	
MIND	Threat	5	4	3	2	1	0
LTH	Threat	26+	21-25	16-20	11-15	06-10	0-5
TE	Threat	0	01-05	06-10	11-15	16-20	21+
AP	Threat	0-5	06-10	11-15	16-20	21-25	26+

NBS: number of squares crossed; MD: mean distance between the heifer and the stimulus in squares (one square = 1.5m); MIND: minimum distance between the heifer and the stimulus; LTH: Latency time before interacting with human; TE: latency time before eating; AP: time spent at less than 1m from the stimulus.

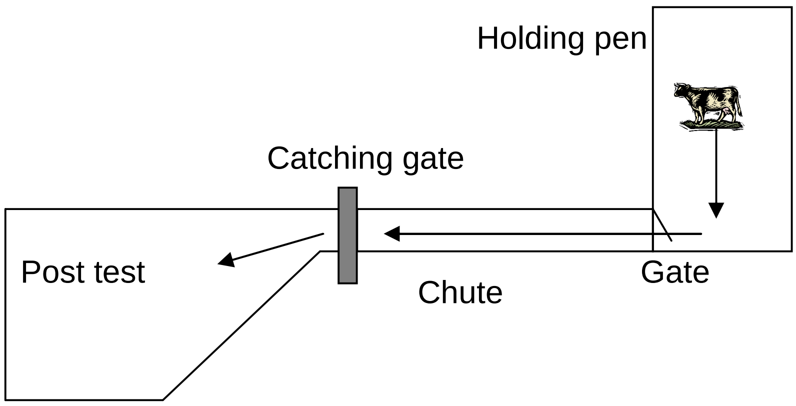

Figure 4. Chute test Procedure: The heifers were removed from their home pen to a holding pen and were not mixed with unfamiliar animals. The animals were weighed and held in another holding pen. They were led to chute where they were restrained with a head catching gate while being blood sampled. Once in the catching gate, the animal was left 5 seconds. After the 5 seconds had elapsed, the animal was handled for 5 seconds before a jugular clamp was used to assist with blood sampling. The heifer was then released to a post test pen.

Table 4. References used to assign scores to ease of entering the chute and ease of restraining the animals during the chute test.

Scores	**Reference**
Willingness of entering	
3	The animal enters willingly
2	The animal pauses before entering
1	The animal pauses and is pushed to enter the chute
Ease of restraining	
4	The animal is easily restrained (no struggle)
3	The animal makes a few smooth head movements
2	The animal makes many strong head movements
1	A nose clamp is necessary to restrain the animal

Table 5. References used to assign scores to the measurements of the chute test variables.

Score	**1**	**2**	**3**	**4**
ENT	1	2	3	
RES	1	2	3	4
AGIT	12+	8-11	4-7	0-3
SPE	≤1	1.1-1.5	1.6-2.0	2.1+

The higher the score, the quieter the animal. ENT: easiness to make the animal enter the chute; RES: easiness to handle the animal at the catching gate; AGIT: overall agitation of the animal during blood sampling; SPE: speed of exit in seconds.

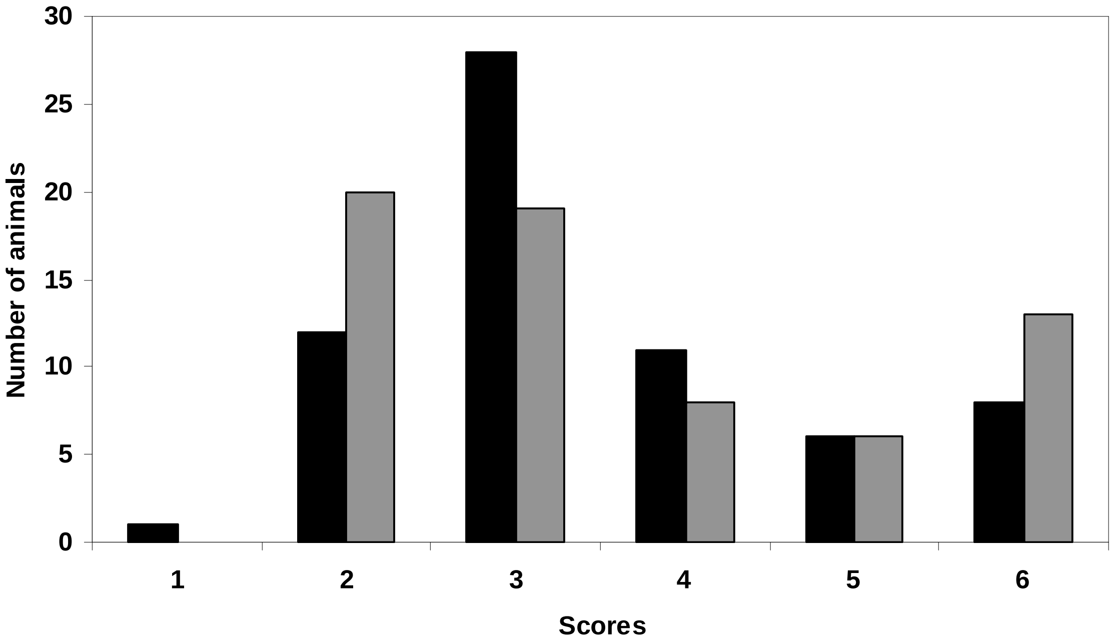

Figure 5. Scores assigned to the heifers for the latency time to join peers. ■: latency time during the first session; ■: latency time during the second session. The higher the score the less the animals were fearful of isolation and took time to join the peers.

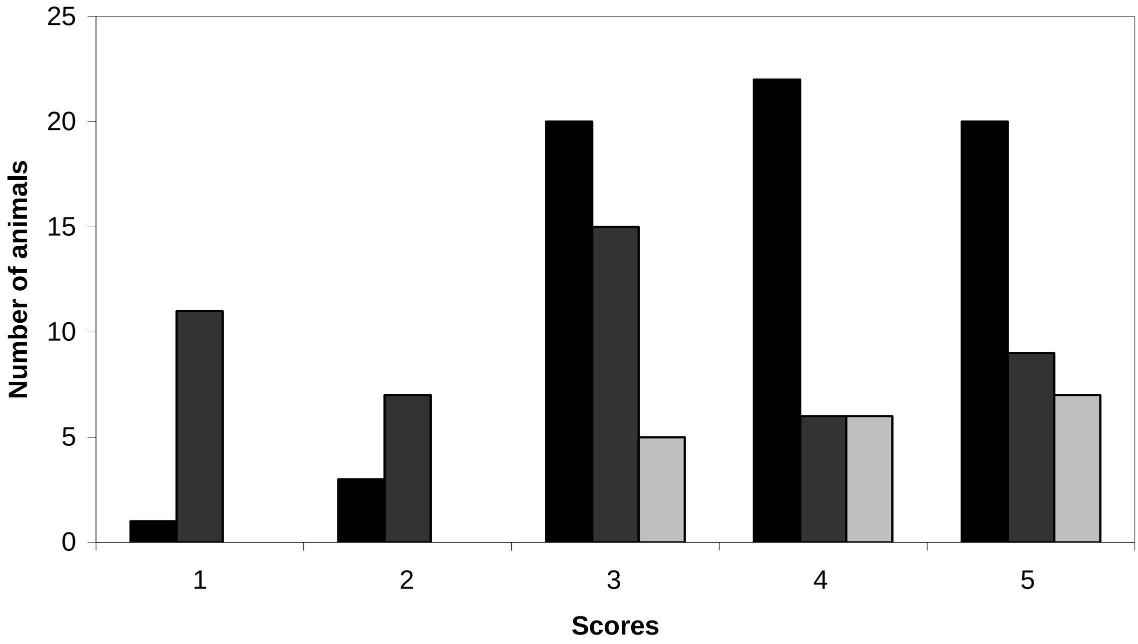

Figure 6. Scores assigned to the heifers for the avoidance distance. : avoidance distance before flight during the first session; : avoidance distance before flight during the second session for PB; : avoidance distance before flight during the second session for CB The higher the score the less the animals were fearful of an approaching human.

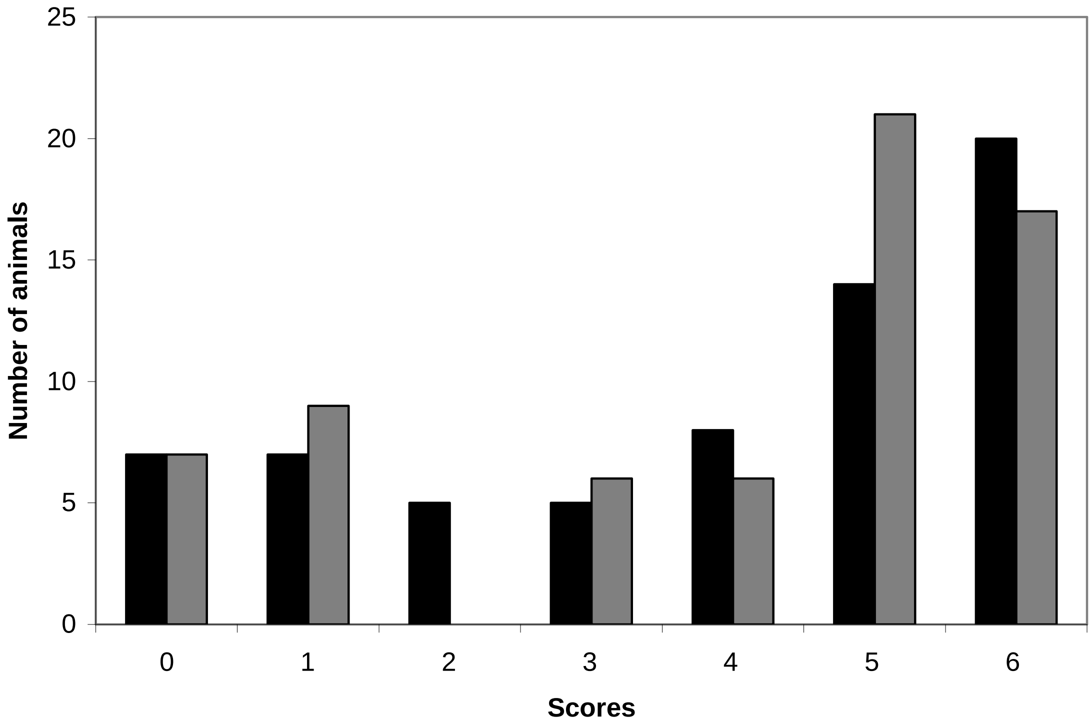

Figure 7. Distribution of the scores of the docility test. : time needed to lead the animals into the corner of the pen during the first test session; : time needed to lead the animals in the corner during the second test session. The higher the score the easier the animal responded.

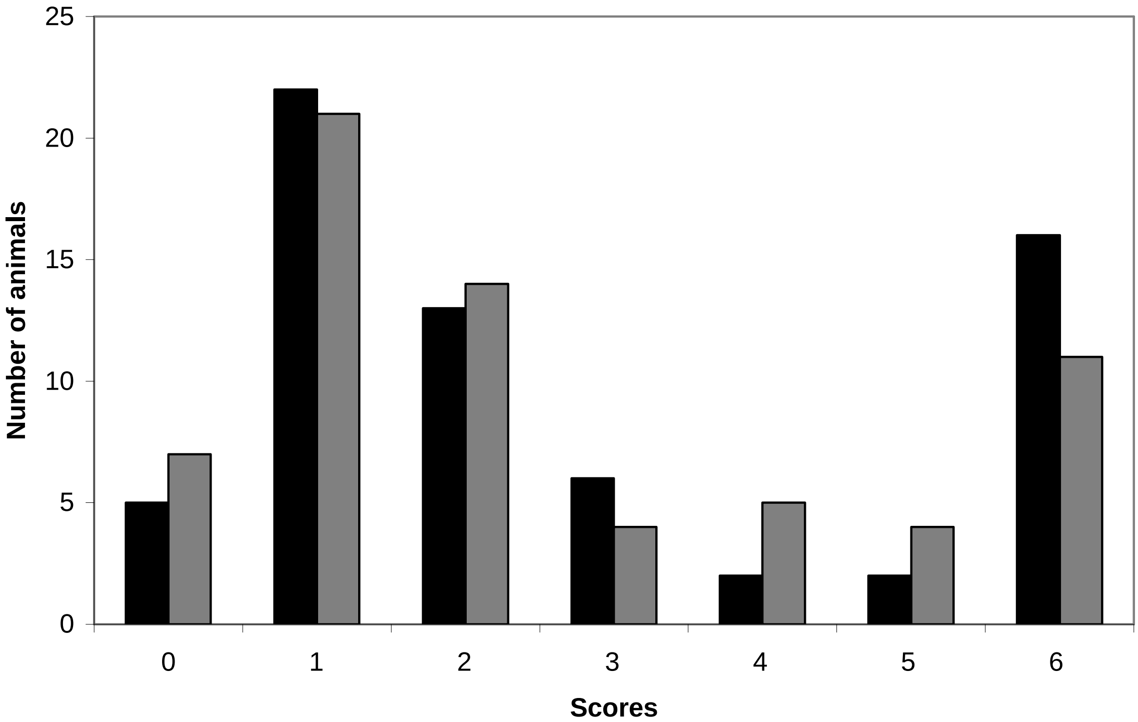

Figure 8. Distribution of the scores of the docility test for the time the heifers could be restrained in the corner.: time the animals stayed in the corner during the first session; : time the animals stayed in the corner during the second session. The higher the score the easier the animal responded.

Table 6. Results of the fear responses for each phase of each session of the fear test.

Phases	1-1	2-1	3-1	4-1	1-2	2-2	3-2	4-2
NBS	3.3 ± 0.1^{a}	$4.4 \pm 0.1^{b***}$	$4.3 \pm 0.2^{b*}$	$4.5 \pm 0.2^{b***}$	3.4 ± 0.1^{a}	3.5 ± 0.2^{a}	3.6 ± 0.3^{a}	$3.7 \pm 0.3.1^{a}$
MD		$2.9 \pm 0.1^{b***}$	1.7 ± 0.1^{a}	$3.2 \pm 0.2^{c*}$		1.6 ± 0.2^{a}	1.5 ± 0.2^{a}	2.7 ± 0.2^{b}
TE		$2.1 \pm 0.2^{b***}$	$1.1 \pm 0.1^{a*}$	$1.2 \pm 0.1^{a***}$		1.1 ± 0.1^{b}	1.0 ± 0.1^{b}	0.1 ± 0.1^{a}
LTH			1.4 ± 0.2^{a}	$2.7 \pm 0.3^{b**}$			1.8 ± 0.2^{a}	1.7 ± 0.2^{a}
AP		$4.1 \pm 0.2^{b***}$	2.1 ± 0.2^{a}	$5.0 \pm 0.2^{c***}$		2.3 ± 0.3^{a}	2.3 ± 0.3^{a}	4.0 ± 0.3^{b}

NBS: mean number of squares crossed; MD: mean distance from stimulus; TE: mean time eating; LTH: mean latency time to interact with human; AP: mean time spent at less than one box (1.5m) from the stimulus. Results are given ± s.e.m; ^{a,b,c}: significant differences between phases of a same session (RemL procedure for repeated measures, least significant differences: P ≤ 0.05%); *, **, ***: significant differences between the same phase of each session (Wilcoxon matched-pairs test, N=71); ***: P<0.001; **: P≤0.01; *: P≤0.5. The first numbers of the phases correspond to the phases of the test (1, 2 3 or 4), the second ones to the session (1 or 2).

Table 7. Correlations between the variables two sessions of the fear test.

Variables	R_s	P-value
NBS1	NS	0.42
NBS2	NS	0.87
NBS3	0.39	0.05
NBS4	0.68	<0.001
MD2	NS	0.66
MD3	0.33	0.04
MD4	0.39	0.05
TE2	0.44	0.01
TE3	0.60	0.02
TE4	0.79	0.009
LTH3	NS	0.35
LTH4	0.34	0.02
AP2	NS	0.63
AP3	NS	0.16
AP4	0.54	0.003

NBS: number of squares crossed; MD: mean distance from stimulus; TE: time eating; LTH: latency time to interact with human; AP: time spent at less than one box (1.5m) from the stimulus. Correlations were calculated with a Spearman's rank correlation test, N = 71 the numbers (1, 2, 3 or 4) correspond to the phase of the test.

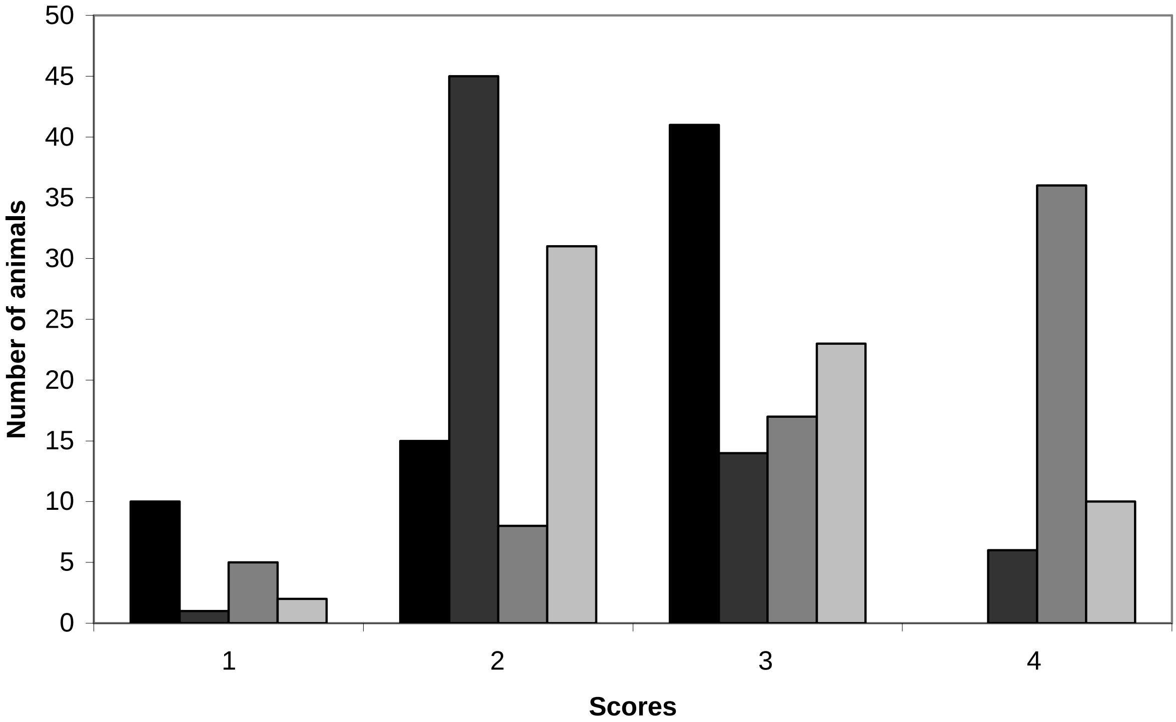

Figure 9. Distribution of the scores of the chute test. ■: willingness to enter in the catching gate; ■: easiness to handle the animals while being blood sampled; ■: overall agitation of the animals during the blood sampling process; □: speed of exit. Higher scores represent quieter animals.

Table 8. Correlation matrix obtained with the PCA between all the variables of all tests.

	TC	**TR**	**RES**	**ENT**	**AGIT**	**SPE**	**NB1**	**NB2**	**NB3**	**NB4**	**MD2**	**MD3**	**MD4**	**TE2**	**TE3**	**TE4**	**AP2**	**AP3**	**AP4**	**LTH3**	**LTH4**	**LAT**	**DIST**
TC	1.00																						
TR	**0.53**	1.00																					
RES			1.00																				
ENT				1.00																			
AGIT	**-0.29**	**-0.38**	**0.28**	**0.24**	1.00																		
SPE		**0.27**				1.00																	
NB1	**0.35**	**0.31**					1.00																
NB2	**0.30**						**0.81**	1.00															
NB3	**0.54**	**0.33**					**0.80**	**0.78**	1.00														
NB4	**0.53**	**0.33**					**0.78**	**0.79**	**0.97**	1.00													
MD2							**0.52**	**0.38**	**0.42**	**0.46**	1.00												
MD3	**0.43**						**0.47**	**0.53**	**0.64**	**0.62**	**0.36**	1.00											
MD4	**0.52**	**0.29**					**0.64**	**0.67**	**0.86**	**0.92**	**0.43**	**0.60**	1.00										
TE2	**0.36**	**0.32**					**0.49**	**0.46**	**0.49**	**0.51**	**0.56**	**0.53**	**0.49**	1.00									
TE3	**0.36**	**0.24**					**0.41**	**0.40**	**0.53**	**0.53**	**0.46**	**0.68**	**0.51**	**0.80**	1.00								
TE4	**0.40**	**0.24**					**0.27**		**0.35**	**0.34**	**0.25**	**0.38**	**0.38**	**0.52**	**0.45**	1.00							
AP2	**0.24**						**0.40**	**0.45**	**0.50**	**0.55**	**0.70**	**0.28**	**0.54**	**0.54**	**0.39**	**0.25**	1.00						
AP3	**0.39**						**0.36**	**0.46**	**0.56**	**0.56**	**0.35**	**0.88**	**0.55**	**0.41**	**0.59**	**0.38**	**0.24**	1.00					
AP4	**0.55**	**0.30**					**0.63**	**0.67**	**0.85**	**0.90**	**0.40**	**0.58**	**0.97**	**0.43**	**0.43**	**0.37**	**0.53**	**0.52**	1.00				
LTH3								**0.34**	**0.41**	**0.42**		**0.55**	**0.45**	0.10	**0.31**	**0.26**		**0.59**	**0.43**	1.00			
LTH4							**0.30**	**0.42**	**0.42**	**0.47**		**0.32**	**0.47**	**0.24**			**0.30**	**0.40**	**0.47**	**0.44**	1.00		
LAT						**0.28**																1.00	
DIST	**0.28**	**0.34**					**0.40**	**0.44**	**0.41**	**0.44**	**0.25**	**0.48**	**0.42**	**0.36**	**0.37**			**0.50**	**0.41**	**0.30**	**0.34**	**0.35**	1.00

TC: time to lead the heifer in the corner; TR: time the heifer was contained in the corner; RES: ease of restraining at the catching gate; ENT: voluntarism to enter the race; AGIT: general agitation; SPE: speed of exit from the chute; NBS: number of boxes crossed; MD: mean distance from stimulus; TE: time eating; AP: time spent at less than 1.5m from stimulus, LAT: latency time to join the peers (flight test). The numbers following the variables represent the phase of the test (1, 2, 3 or 4). LAT: latency time to join the peers; DIST: avoidance distance Bold numbers represent significant correlations (Principal component analysis, N = 66, P ≤ 0.05).

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