WHAT ARE THE EMOTIONAL RESPONSES OF TIE-STALL COWS TO INDOOR AND OUTDOOR EXERCISE ACCESS?

BY

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ABSTRACT

The status of animals has changed quite recently in the province of Quebec, such that they are now considered as sentient beings, which means that the law now considers animals capable of emotions. Animals should thus be properly cared for, and to ensure good welfare, we should now not only consider their physical state, but also their psychological state. Our literature review showed different types of tests commonly used to study emotions: arena, human, novel object and frustration/excitement tests. Most of the tests looking at emotions focus on the negative ones (e.g., fear of humans).

The objective of our study was to look at the effect of indoor and outdoor exercise access on the emotions of tie-stall-housed dairy cows. To be able to answer this, three different objectives were defined: 1- Determine the cow's motivation to go to the outdoor or indoor exercise area; 2- Determine the influence of the outing on the cow's reactivity to (i) a human, (ii) a sudden event, (iii) novelty, after one period of treatment application; 3- Determine the influence of the overall experience on aspects studied in objective 2. Twenty-seven lactating Holstein cows were used in a repeated 3x3 latin square for three periods of three weeks following a habituation period of eight days. Cows underwent one of the three treatments each period: 1- No exercise access (in home stall continuously); 2- Outdoor exercise access; 3- Indoor exercise access. The treatments including exercise access were applied for one hour per day, for two consecutive days each week. For objective 1, the cow's motivation to go in the outdoor or indoor exercise area was measured by the evolution over time of the speed to access either exercise area: thus, the time taken to put on the halter, the trip duration and the total duration for the moving between the stall and the exercise area were measured. For objectives 2 and 3, all cows were observed live in their tie-stall in a human approach test for which the reaction of the cow and her latency to first touch the person were measured. Cows were also video recorded in a suddenness test for which their reaction and latency to touch the pin and to regain normal activity were measured. Cows were also video recorded in a novel object test where their behaviors before and after the fall of a ball were observed. After the fall, behaviors related to the novel object, distances kept from the novel object and behaviors not related to the object were observed. The results from objective 1 show that the time to put on the halter decreased and speeds (trip and total) increased over periods of treatment application ($P < 0.05$), reflecting the

cows habituating and learning to go to the exercise areas. A treatment effect was found for the total speed ($P < 0.0001$, Ddf = 54.8, F-value = 30.84), with outdoor treatment cows being faster, thus probably more motivated than indoor treatment cows. The handler had a significant impact on the time to put on the halter on the cows for the trip test ($P = 0.0001$, Ddf = 49.6, F-value = 17.83). The results from objective 2 were not as expected: we found very few treatment*time effects after the first period of treatment application. This may indicate that cows were not exposed enough to the different physical environments, procedures and handling (i.e., treatments). An important finding from the novel object test was a significant time effect for many variables showing that cows became habituated to the novel object test across the 1-month of trial. For our objective 3, after the overall experience, we found a significant time effect ($P < 0.05$) for most of human test variables, with an increase in scores over time reflecting of cows approaching humans more during the test. No improvement in reactivity to suddenness was observed (no time effect; $P > 0.05$). After the overall experience on the novel object test, we found a significant time effect for a number of variables, such as frequencies of looking, approaching and moving away from the object, which decreased over time ($P < 0.05$). Notably, the time effect was found non-significant for the time spent interacting with the object and the time spent looking at the object ($P > 0.05$). Our novel object test results show that it is possible to arouse curiosity with six weeks of provision of indoor or outdoor exercise access.

This research study provides new information supporting the provision of exercise access to dairy cows, by showing motivation and curiosity elicited by exercise access in these animals. Additionally, the positive relationship between cows and humans resulting from this experiment is beneficial for both species. Exercise access provision can decrease cows' fear reactions and facilitate handling for day-to-day routine.

RÉSUMÉ

Le statut juridique des animaux a changé récemment au Québec, si bien que ces derniers sont désormais considérés comme des êtres sensibles, capables d'émotions. Ainsi, ces derniers devraient recevoir des soins appropriés, et, pour assurer leur bien-être, nous devrions nous concentrer non seulement sur leur état physique, mais aussi sur leur état psychologique. Notre revue de littérature a présenté différents types de tests utilisés pour étudier les émotions : les tests de l'arène, de l'homme, du nouvel objet et de la frustration/excitation. La plupart des tests effectués se concentrent sur les émotions négatives (exemple : peur de l'homme).

L'objectif de notre étude était d'observer l'effet de l'accès à l'exercice en intérieur ou en extérieur sur les réponses émotionnelles des vaches logées en stabulation entravée. Trois objectifs distincts ont été définis : 1- déterminer la motivation de la vache à se rendre à l'aire d'exercice intérieure ou extérieure 2- déterminer l'influence de l'accès aux aires d'exercice sur la réactivité de la vache à (i) l'homme, (ii) un évènement soudain, (iii) la nouveauté, suite à une période d'application des traitements 3- déterminer l'influence de l'expérience globale sur les aspects étudiés dans l'objectif 2. Vingt-sept vaches Holstein en lactation ont été utilisées dans le cadre d'un carré latin répété 3x3 de trois périodes de trois semaines, suivant une période d'habituation de huit jours. Les vaches ont reçu chaque période l'un des trois traitements : 1- aucun accès à une aire d'exercice (en stalle en continu); 2- accès à l'aire d'exercice extérieure; 3- accès à l'aire d'exercice intérieure. Les traitements incluant un accès à une aire d'exercice étaient appliqués à raison d'une heure par jour pendant deux jours consécutifs chaque semaine. Pour l'objectif 1, la motivation de la vache à se rendre à l'aire d'exercice intérieure ou extérieure a été mesurée via l'évolution de sa vitesse pour s'y rendre : ainsi, le temps requis pour la pose du licou, la durée de trajet et la durée totale de ces deux étapes entre la stalle et l'aire d'exercice ont été notés. Pour les objectifs 2 et 3, toutes les vaches ont été observées en direct dans leur stalle pour le test à l'homme, où leur réaction à l'approche d'un humain et leur latence à le toucher ont été mesurées. Les vaches ont aussi été filmées pour le test de soudaineté, où leur réaction et leur temps passé figé lors de la chute d'un objet ont été mesurés. Les vaches ont aussi été filmées pour le test du nouvel objet où leur comportement avant et après l'apparition d'un objet nouveau (ballon coloré) a été observé. Les comportements liés au ballon, les distances par rapport à cet objet et les comportements indicateurs de confort ont été relevés. Les résultats de l'objectif 1 montrent une diminution du temps de pose du licou et une augmentation des vitesses (trajet et total) au cours des périodes de traitement ($P < 0.05$), démontrant que les vaches se sont habituées aux procédures et ont appris à se rendre aux aires d'exercice. Un effet significatif du traitement a été obtenu pour la vitesse totale ($P < 0.0001$, DDL = 54.8, $F = 30.84$), les vaches allant à l'extérieur étant plus rapides, probablement parce qu'elles étaient plus motivées que les vaches du traitement intérieur. Le manipulateur a eu un impact significatif sur le temps de pose du licou sur les vaches pour le test du trajet ($P = 0.0001$, DDL = 49.6, $F = 17.83$). Pour l'objectif 2, contrairement à ce qui était attendu, nous avons obtenu très peu d'effet traitement*temps après la première période d'application des traitements. Cela pourrait indiquer que les vaches n'avaient pas été suffisamment exposées aux traitements, c'est-à-dire aux différents environnements physiques, procédures et manipulations. Pour ce qui est du test du nouvel objet, l'effet significatif du temps obtenu pour plusieurs variables montre que les vaches se sont habituées au test au courant d'un mois d'expérience. Pour l'objectif 3, soit suite à l'expérience entière, nous avons trouvé un effet temps significatif pour la plupart des variables du test à l'homme ($P < 0.05$), avec une augmentation des scores avec le temps (c'est-à-dire que les vaches approchent l'homme plutôt que de l'éviter durant le test). Aucun effet temps n'a été obtenu pour le test de soudaineté ($P > 0.05$), indiquant qu'il n'y a eu aucune amélioration de la réactivité à la soudaineté. Suite à l'expérience globale pour le test du nouvel objet, nous avons identifié un effet temps significatif pour plusieurs variables, dont les fréquences de regard, d'approche et de recul face à l'objet, qui ont diminué avec le temps ($P < 0.05$). Aucun effet temps significatif n'a été obtenu pour le temps passé à interagir avec l'objet et le temps passé à regarder l'objet ($P > 0.05$). Ainsi, nos résultats sur le nouvel objet montrent qu'il est possible de générer de la curiosité, en leur procurant ne serait-ce que six semaines d'accès à l'exercice intérieur ou extérieur.

Cette étude procure de nouvelles informations en soutien à l'accès à l'exercice pour les vaches laitières, en montrant la possibilité de procurer aux vaches de la motivation et de la curiosité. De plus, une relation positive entre celles-ci et l'humain résultant de cette expérience est bénéfique pour tous deux. L'accès régulier à l'exercice peut permettre de réduire les réactions de peur chez les vaches et faciliter leur manipulation au quotidien.

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CHAPTER 1 – GENERAL INTRODUCTION

Until quite recently, animals have been considered by law as movable property. In 2015, the status of animals has changed in the province of Quebec and they are now considered as sentient beings, which means that the law considers animals capable of emotions (LégisQuébec, 2021). Research showed that animals have emotions, which are defined as “an intense but short-living affective response to an event […] and is materialized in specific body changes” (Désiré et al., 2002). Knowing that animals are capable of emotions, proper care should be provided to them. In order to ensure good welfare, we should not only take into account their physical state but also their psychological state (Veissier et al., 2012). Knowing their emotions can confirm the impact of an experience on them and this can help to reach better welfare. Understanding how animals communicate their emotions then becomes essential (Battini et al., 2019).

Emotions can be measured on a continuum along two axes: valence (either positive or negative) and arousal or level of excitement (either high or low) (Mendl et al., 2010). Fear is one emotion often studied in research (Forkman et al., 2007). In general, exposure to suddenness, novelty and humans are three different aspects commonly used to investigate reactivity (Désiré et al., 2002). Reactivity is defined as the capacity to perceive and react to potentially anxiogenic situations (Boissy, 1995). Behavioral tests, which are tests often experimentally used on events that animals can face in farming, are used to investigate different types of emotions (Désiré et al., 2002). Novel object test, suddenness test and arena test are some examples. However, for each type of test, there are differences in design, duration and recordings from one study to another (Boissy and Bouissou, 1995).

In Canada, 73 % of barns have a tie-stall system (CDIC, 2020). In tie-stall systems, cows are deprived of voluntary movement and exercise access during most of their lives (Shepley et al., 2020). The National Farm Animal Care Council considers access to exercise of indoor systems cows as a priority, being listed in the priority welfare issues for the Scientific Committee report (NFACC, 2021) and the on-going revision of the Code of practices will most likely require some form of exercise access in tie-stall barns. The objective of the experimental study is to define how to provide exercise by reorganizing indoor and outdoor spaces and to understand cows’ motivation and reactivity in relation to this exercise experience. Solutions to cows’ lack of access to exercise could now be perceived positively for both the animal and the human.

CHAPTER 2 – A REVIEW OF THE TESTS CURRENTLY USED TO EXAMINE BOVINES' EMOTIONS

2.1. METHODS

In recent years, there has been a growing interest in animal welfare. Concern on this topic is highly associated with the attribution of mental states to animals (Désiré et al., 2002). An emotion can be defined as "an intense but short-living affective response to an event […] and is materialized in specific body changes" (Désiré et al., 2002). Emotions can be measured on a continuum along two axes: valence (either positive or negative) and arousal or level of excitement (either high or low) (Mendl et al., 2010). Valence corresponds to the animal's perception of the experience as positive or negative, rewarding or punishing, and pleasant or unpleasant (Mendl et al., 2010). Fear, for example, results from a negative valence and high arousal and is defined as the reaction to the perception of actual danger (Forkman et al., 2007; Proctor and Carder, 2014). The latter is the most commonly investigated emotion in domestic animals and tests related were reviewed by Forkman et al. (2007). Research also shows that different emotions can be experienced by bovines, such as frustration and excitement. However, based on Désiré et al. (2002)'s concept, there might be more which are still unknown.

In order to study the emotions of bovines, different measures are used. Observation of behaviors is common. For example, walking and running are observed to describe locomotion and sniffing and approach are observed to describe exploration behavior. The observation of body postures, such as those of the ears, are also helpful. Additionally, physiological measurements of the heart rate, hormones (e.g. cortisol) and eye whites are used. Measures can be taken either during live observations in normal living conditions or during behavioral tests (Battini et al., 2019). The latter often experimentally replicate events which animals can face in farming conditions (Désiré et al., 2002). The objective of this scoping review is to describe literature related to the tests used to examine dairy cows' emotions. The question related to this objective thus is: what are the tests currently used to examine bovines' emotions?

2.1.1. Protocol

The protocol used in this review is the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Extension for Scoping Reviews (PRISMA-ScR, Tricco et al., 2018).

2.1.2. Eligibility criteria

In order to be included in this review, articles had to follow several criteria. First, papers had to discuss female dairy bovines (or a mix of females and males) and tests triggering an emotion to them. Ideally, papers mentioning measures that could help detect the emotion felt by the cow were selected. To be included, the article had to be published after 2000. One exception was made for one article published in 1975 which was considered relevant for this review. Additionally, publications had to be peer-reviewed, exclusively in English and containing the full text. Review, conference, as well as thesis and symposium papers were excluded, even though tags were added. One congress paper was included because of its relevance. Tags were also added to on-farm assessment papers. The same was done with papers talking about beef, crossbred cows, production/performance/longevity, milking temperament, temperament traits and humans.

2.1.3. Information sources and search strategy

The following databases were used for this research: Scopus, Web of Science and CAB Abstracts. The main concepts are related to dairy cows and emotions or affective states or feel* or temperament. The Boolean (AND, OR, W/15) and truncation (*) were used. The same search strategy was used for all of the databases and no limit was set in them (see Table 2.1.1). The last research was conducted on June 10th, 2020.

Table 2.1.1. List of different strings included in the search strategy and number of records associated with each string. Application on Scopus database on June 10th 2020.

Query #	String	Records
1	TITLE-ABS-KEY(dairy W/15 (cow OR cattle OR calf OR calves OR heifer OR bovine))	55 294
2	TITLE-ABS-KEY((emotion* W/15 state) OR (emotion* W/15 react*) OR (emotion* W/15 respon*) OR (emotion* W/15 test) OR (emotion* W/15 assess*) OR (emotion* W/15 measur*) OR (emotion* W/15 analy*) OR (emotion* W/15 evaluat*) OR (emotion* W/15 experience) OR (indicat* W/15 emotion*))	179 510
3	TITLE-ABS-KEY((affective W/15 state) OR (affective W/15 react*) OR (affective W/15 respon*) OR (affective W/15 test) OR (affective W/15 assess*) OR (affective W/15 measur*) OR (affective W/15 analy*) OR (affective W/15 evaluat*) OR (affective* W/15 experience))	47 031
4	TITLE-ABS-KEY((feel* W/15 react*) OR (feel* W/15 respon*) OR (feel* W/15 test) OR (feel* W/15 assess*) OR (feel* W/15 measur*) OR (feel* W/15 analy*) OR (feel* W/15 evaluat*) OR (feel* W/15 experience) OR (indicat* W/15 feel*))	62 102
5	TITLE-ABS-KEY((temperament W/15 react*) OR (temperament W/15 respon*) OR (temperament W/15 test) OR (temperament W/15 assess*) OR (temperament W/15 measur*) OR (temperament W/15 analy*) OR (temperament W/15 evaluat*) OR (temperament* W/15 experience) OR (indicat* W/15 temperament))	6 942
Combined queries	#1 AND (#2 OR #3 OR #4 OR #5)	123

2.1.4. Selection of sources of evidence

All of the articles resulting from the research (479) were imported into Covidence to remove duplicates and for the screening process. An exclusion was first done after screening the title and abstract from every reference. Also following the inclusion and exclusion terms mentioned above, other articles were excluded following a full-text screen. Finally, one article was referring to two other studies that were relevant, and were searched in the grey literature afterwards. The whole process was performed by a single person.

2.1.5. Data charting process and data items

After the screening process, the remaining articles were charted using Excel software (Version 16.30). The extraction was performed by a single person. The publications were charted as follows: author, year, title, location, journal, research aim, duration of the study and recording intervals, number of farms, description of the population (sample size, breed, sex, age and production cycle), animals' groups/intervention/treatments, type of test, name of test, general description of the test and time, behavioral and physiological measures and time, technology used, validation of the test and measure, results, conclusion and study limitations.

2.1.6. Synthesis of results

The data extracted in the Excel chart was used in other charts classified by tests. Each test had a chart adapted to its specific conditions. These were later used to summarize the information on each test and make comments on them. The general information reported included the animal used (size, breed, sex), the description of the location where the test was performed, the description of the test and the time, the number of times the test was performed and the time intervals, and the behavioral and physiological measurements.

2.2. RESULTS

2.2.1. Selection of sources of evidence

A total of 481 papers resulted from the literature search done on Scopus (n = 123), Web of Science (n = 181), CAB Abstracts (n = 175) and from other sources (n = 2) (see Figure 2.2.1). After the duplicates were removed, 282 articles were remaining. During the title and abstract screening, 220 records were excluded leaving 62 articles. Those 62 publications were assessed for full-text screening and 36 of them were removed for different reasons. Some of them were excluded because they were either a book, a review, a thesis or an unpublished paper. Others were excluded because they were not using the right population, were not looking at tests on emotions or were only looking at temperament. Most of them were excluded because they were looking at the wrong tests on emotions. Finally, 2 publications were not in English and there were still 2 duplicates. A total of 26 papers remained at the end of the whole process, which were used in the scoping review.

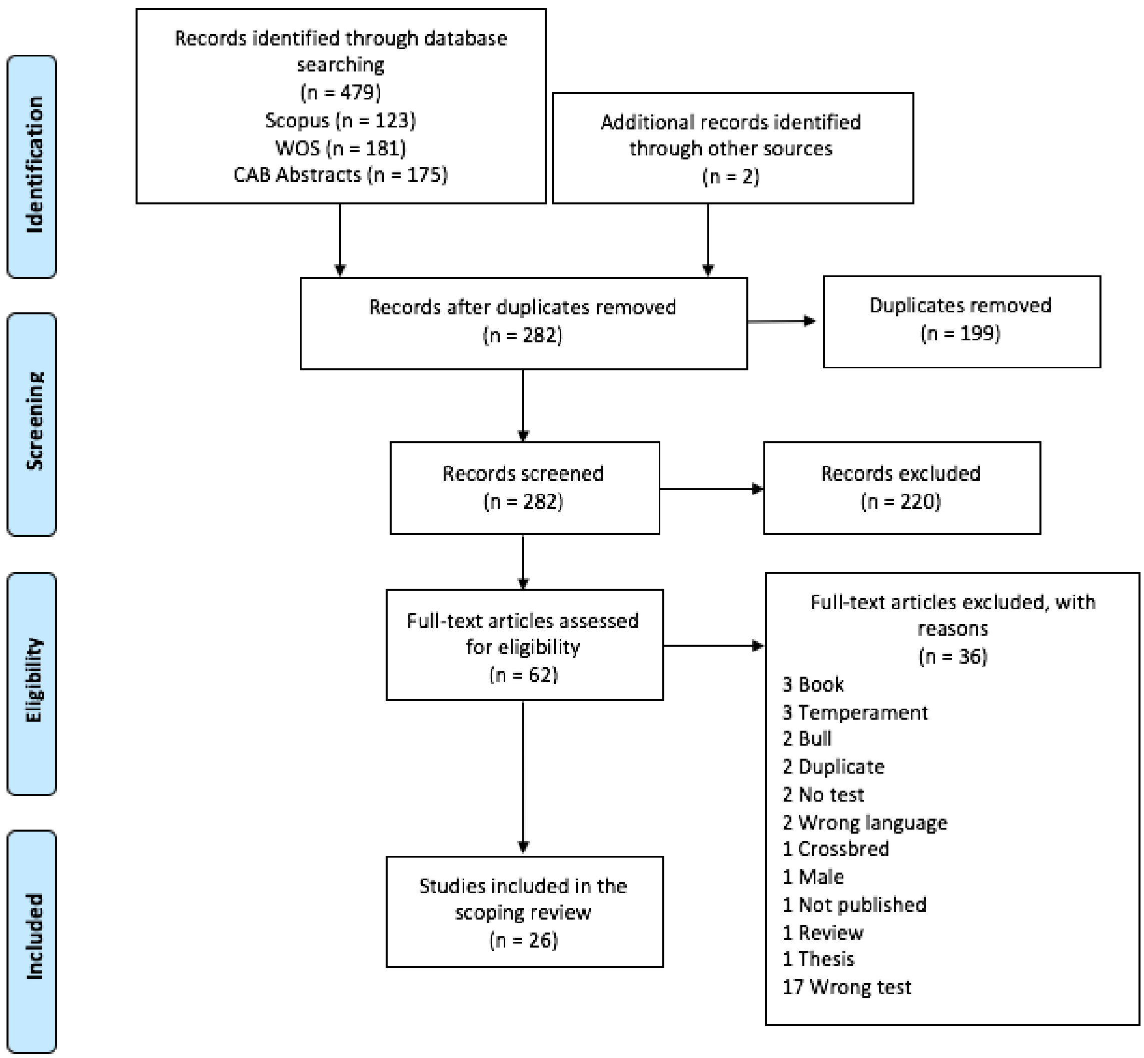

Figure 2.2.1. PRISMA flow diagram illustrating the different steps followed for the selection and retention of literature.

2.2.2. Overview of sources

Among the 26 studies included in this scoping review, the human test was the most common test (54 %) and the arena one, the least (19 %). Furthermore, 73 % of the articles used at least one of the tests normally designed to examine fear (arena, novel object and human tests). The 26 papers used in the present review are not particularly recent. In fact, one article performing the arena test was published in 1975 (Kilgour, 1975). Otherwise, newer research was performed with the judgment bias test (Lecorps et al., 2019). This type of test is different from the others (arena, human, novel object and frustration/excitement tests) since it is looking at mood rather than emotions. Moods are long-lasting emotional states which are not only a response to one event, but an accumulation of different emotions to different events (Proctor and Carder, 2014). They can occur in the absence of a direct stimulus (Proctor and Carder, 2014). Instead, emotions tend to be short and immediate reactions, are usually event-focused and occur only in response to a direct positive or negative experience (Proctor and Carder, 2014). Articles on play behavior, motivation and separation with calf were also excluded, since only a few studies approached these subjects. In behavioral tests, more than half of the papers (65 %) used cows instead of heifers and calves. Often, this will depend on the question asked by the authors. Calves are more commonly used in studies looking at pain (e.g. disbudding) and play, since this behavior and this procedure are more common with young animals.

Few authors report the validity aspects of their test in their research. However, they are well presented in the Forkman et al. (2007) critical review of fear tests (novel arena, novel object and handling tests). Additionally, individual animal factors such as genetics, previous experience and others were either briefly or not discussed in papers. Experimental designs were not set up to ask this kind of question. Boissy et al. (2007) partly talk about these factors in their review. However, temperament is a common subject and was studied for genetic evaluation, but this is not the objective of this scoping review. Thus, validity and individual animal factors are not discussed here.

The present scoping review focuses on the arena, human, novel object and frustration/excitement tests. Each is described by presenting aspects in the following order: aim (and specifically, which emotion is targeted), environment where the test is performed, conditions before and during the test and measurements. The discussion will focus on factors to consider when planning these tests and the limits of each of them.

2.3. TESTS

2.3.1. Arena test

The aim of the arena test, also called the open field test or the novel environment test is to assess the fear of novelty (Boissy and Bouissou, 1995; Van Reenen et al., 2004). This test consists of putting an animal in a novel environment for a small duration of time. It is also possible to use an arena test to examine positive emotions associated with play behavior using a different process, but this kind of test will not be part of the discussion in this review (Rushen and de Passillé, 2012; Rushen et al., 2016).

The size of the arena varies from 16 m^2 to 36 m^2. A square form was mentioned in the case of Van Reenen articles (Van Reenen et al., 2004; Van Reenen et al., 2005; Van Reenen et al., 2013). Depending on the experiment, some scientists are using different arena sizes varying with the age of the animal. For example, Van Reenen et al. (2004) built an arena of 16 m^2 for 3 week-old calves, followed by 20 m^2 and 36 m^2 arenas for calves of 16 and 29 weeks-old, respectively. As mentioned earlier, the arena is unfamiliar to the animal. It is also located either inside or outside and is built on a concrete floor, whether covered or not. The arena is made of walls or panels of approximately 2 meters high each.

In order to keep it novel, the animal is not exposed to the arena prior to the first test. Since the arena test is not performed in the home environment, it includes transportation. The distance to travel can vary between 20 to 40 m (Van Reenen et al., 2004). In general, a human would gently lead the animal to the arena, using a halter or not. The animal would sometimes have had previous experience if it had been manipulated this way before during a habituation period. Van Reenen et al. (2004) and Kilgour (1975) used different methods. In the first article, transport by a familiar wheeled cart was used. Also, the calves had the chance to be briefly transported on it three times before the experiment began. In the second one, the authors developed external raceways where the cows would travel to the entry gate of the outdoor arena. This procedure reduces the presence of humans during transportation. In Van Reenen's experiments (Van Reenen et al., 2004; Van Reenen et al., 2005), they first confined the calves individually in a start box for 3 minutes after transportation as an acclimation period.

Once the animal is in the arena, the calf or cow is left alone for 5 to 15 minutes. During the test, observers might be present to do live observations. As it was done by Kilgour (1975), three 3-meter-high observation towers were placed for screening observers for scoring during the 5 testing minutes.

Measures. Most of the observations made in this test concern behaviors. The majority are related to locomotion and vocalizations (see Table 2.3.1). Some are also looking at elimination, exploration and latency to enter the arena. The behavioral observations are normally measured during the whole time the animal is in the arena. Very few experiments included physiological measurements to observe the reaction of the cows to the arena test. In the case of the articles found, two took blood samples for plasma cortisol concentrations (before and after the test) and measured heart rate (continuous sampling during three days before and during the test) (Van Reenen et al., 2005; Van Reenen et al., 2013).

In summary, mostly calves are used in the arena test. The latter consists in leading them individually into a novel environment varying in size for a small amount of time. Most of the research recorded behavioral observations and few experiments combined them with physiological measurements. Finally, many researchers are also using the arena for human tests, to measure the avoidance distance as it will be presented in the next section.

Table 2.3.1. Behavioral measures reported in arena tests

Reference	Behavioral measures
Lecorps et al. (2018)	Distance covered within the pen
	Time spent exploring
	Number of vocalizations
Van Reenen et al. (2004)	Accumulated times spent in locomotion
	Accumulated times spent in contact (with nose or tongue) with the floor or the walls
	Number of vocalizations
Kilgour (1975)	Number of squares entered during ambulation (ambulation score)
	Number of defecations and urinations (elimination score)
	Number of vocalizations (vocalizations score)
	General behavior
Van Reenen et al. (2013)	Time spent in locomotion
	Number of vocalizations
	Latency to enter the open field
Van Reenen et al. (2005)	Accumulated times spent in locomotion
	Accumulated times spent in contact (sniffing, licking) with the floor or the walls
	Number of vocalizations
	Latency to enter the open field
	Number of defecations and urinations

2.3.2. Human test

The aim of the human test is to observe the animal's fear of people (Van Reenen et al., 2004). Two main types of tests are used: human stationary and human approach tests. In this review, they will be respectively presented from the less intrusive to the most intrusive test. In the first type, the human stays immobile and the animal is offered the choice to approach. In the second, the human is the one approaching the animal.

2.3.2.1. Human stationary

Also called the voluntary approach, this test consists in placing an immobile human in an area and studying the animal reaction to this person (Schütz et al., 2012; Sutherland and Huddart, 2012).

This human test is usually performed in an arena which can vary in size, ranging from 12 to 35 m^2. Van Reenen et al. (2004) adapted the size of the arena to the age of the animals. For example, they made arenas of 12, 20 and 27 m^2 for calves of 3, 16 and 29 weeks-old. Two studies used different environments. Schütz et al. (2012) conducted their experiment in the home pen of the calves. Rushen et al. (1998) performed the test in the home stall and a treatment stall because one of their objectives was to examine if cows could learn to react differently (avoid and approach) towards the same person, depending on whether they were in a location where they had been handled gently or aversively.

When it is not performed in the home environment, the animal has to be transported to the experimental area. Two articles mentioned that they first habituated the animal to transport. In the Van Reenen et al. (2004) study, the calves were briefly transported in a cart to the experimental area on three occasions throughout the week prior to testing. In Rushen et al. (1998), the cows were walked with a halter once every 2 weeks. The type of manipulation to bring the animal to the testing area varies from one article to the other. As mentioned above, a wheeled cart or a halter can be used. Sometimes, the animal is gently directed or quietly moved using only quiet voices and gentle tactile force if necessary (Sutherland and Huddart, 2012; Lecorps et al., 2018).

In general, the animal is alone during the human stationary test. However, Schütz et al. (2012) conducted the experiment with groups of five calves. When it is performed in an arena, the human is already present. In the case of Van Reenen et al. (2004), the human quietly entered the arena following a 3 minutes familiarization period. The human involved in the test is either familiar or unfamiliar. The clothing of this person is not always mentioned, but some studies mentioned overalls of varying colors. Van Reenen et al. (2004) also took care to report their choice of a different overalls color for this person: it was white rather than the green one normally worn by the barn staff. The human is either standing or sitting on a chair. Articles from Sutherland et al. (2012) and Sutherland and Huddart (2012) added that the stationary person sat in a nonaggressive pose with eyes cast down and palms resting on their lap. In another article, it is mentioned that the person was standing still with hands in pockets (Rushen et al., 1998). The main characteristic of the human stationary test is that the person stays still for the whole time. The duration of the test varies between 1 and 10 minutes. Van Reenen et al. (2004) also included a 3-minute familiarization period beforehand. One observer was present during part of the tests conducted by Schütz et al. (2012). Rushen et al. (1998) performed the human stationary test in a home stall or a treatment stall, where a human was 0.5 meters in front of the bar to which the cow was tethered. An observer was also present during the test and stood in front of a neighboring stall in full view of the cow.

Measures. All of the studies included in this review used behavioral measures only (Table 2.3.2). The latencies to approach or contact are the most common. The measures are taken during the entire duration of the test.

In summary, the human stationary test is characterized by a human staying immobile (standing or sitting) in an arena, pen or in front of a stall for a certain amount of time. Only behavioral observations (no physiological measures) were reported in published studies.

Table 2.3.2. Behavioral measures reported in human stationary tests

Reference	Behavioral measures
Van Reenen et al. (2004)	Latencies to approach within 1 m Latency to contact Accumulated times spent in contact Accumulated times spent in locomotion Number of vocalizations
Sutherland and Huddart (2012)	Closest distance of animal's front hooves from the seated person (all 4 hooves stationary for 2 seconds)
Sutherland et al. (2012)	Closest zone, in relation to the seated person, in which the animal stood more than 2 seconds
Lecorps et al. (2018)	Latency to contact Duration of contacts Time spent at proximity Distance covered Number of vocalizations
Schütz et al. (2012)	How many calves approached (calf touching the person with its nose) Latency to approach
Rushen et al. (1998)	Distance the cow kept from the treatment person

2.3.2.2. Human approach

Compared to the human stationary tests, this human test is characterized by a human approaching the animal. Some articles also call it the avoidance distance test or the human avoidance distance (HAD) (Sutherland et al., 2012; Sutherland and Huddart, 2012; Battini et al., 2019).

The studies using the human approach test performed the latter in different locations (Table 2.3.3). Familiarity with the space is not always mentioned. Kovács et al. (2015) made sure the environment was familiar to the cows. They mention that unfamiliar environments could affect the animal's response. Human approach tests are performed either inside or outside. The transport or handling process to the test area is rarely mentioned in papers. In Sutherland and Huddart (2012), one handler was manipulating the animals with quiet voices and gentle tactile force, if necessary.

The human approach test is generally performed individually. Sometimes, other animals might be present in the environment during testing. The person is either familiar or unfamiliar to the animal. The clothes they wear vary from one article to the other. Kovács et al. (2015)

mentioned that the human wore the same clothing during the testing. Additionally, Uetake et al. (2004) used a yellow raincoat, an orange cap and black boots in order to enhance novelty. The main characteristic of this test is the fact that the human approaches the animal, generally in a standardized way. The human stands from the animal at a varying distance of 2 to 10 meters, depending on the article and the location of the test. Different criteria are used to define the start of the test. In some experiments, they made sure the human could make eye contact with the animal. Others, like Kovács et al. (2015) and Uetake et al. (2004), made sure they had the attention of the animal. The approach is usually slow (see Table 2.3.3). During the approach, the person generally does not look directly in the eyes of the animal. Gibbons et al. (2011) looked towards the feet of the cow while Kovács et al. (2015) looked at the muzzle of the animal. In the case of Sutherland et al. (2012) and Sutherland and Huddart (2012), the human kept his eyes casted down. Protocols in regard to the position of the arms differ between studies. Arms and hands can keep close to the body or hands can be rest in the pockets (Gibbons et al., 2011; Kovács et al., 2015). Otherwise, one arm can be lifted at different degrees (45 degrees or 20 to 30 centimeters from body) and the hand palm can be directed downwards (Sutherland et al., 2012; Sutherland and Huddart, 2012; Kovács et al., 2015; Battini et al., 2019). The human can approach until the animal reacts or until he touches the animal. In Uetake et al. (2004), the experimenter kept on going until he reached the point to which the animal was standing when the test started. The overall duration of the test is not mentioned in the papers but will vary with the distance and the methods chosen for the approach (e.g. human pace). Sometimes, an observer is also present during the test. However, their position is never mentioned.

Measures. Mostly behavioral measures are taken for the human approach test (Table 2.3.3). The most popular measure is the distance between the human and the animal at the moment the animal moves away (avoidance distance or flight distance). Few physiological measurements were taken during the human approach test. Battini et al. (2019) looked at the eye whites of the cows to interpret the valence and arousal of emotions in dairy cows. In the case of Kovács et al. (2015), the authors looked at cardiac parameters for their test conducted in the milking parlor.

In summary, this test is characterized by a human slowly approaching the animal. Mostly avoidance distances are measured to investigate fear of humans in this type of test.

Table 2.3.3. Behavioral measures and characteristics of the test (location, speed) reported in human approach tests

Reference	Location of the test	Speed	Behavioral measures
Battini et al. (2019)	Feeding rack	Slowly	Ear postures
Gibbons et al. (2011)	Passageway of the home-pen	Using strides of approximately 0.5 meters and after every step the observer remained motionless for 10 seconds to allow the cow to respond.	Flight response Visual analogue scale (VAS)
Kovács et al. (2015)	Feeding bunk	1 step/s	Avoidance distance Reaction Interaction
	Milking parlor	Not mentioned	None
Sutherland and Huddart (2012)	Paddock	0.5 m/s	Avoidance distance
	Arena (7 x 5 m = 35 m^2)	0.5 m/s	Avoidance distance
Sutherland et al. (2012)	Paddock	0.5 m/s	Avoidance distance
	Arena (7 x 5 m = 35 m^2)	0.5 m/s	Avoidance distance
Uetake et al. (2004)	Pasture	1 m/s (two small steps/s)	Flight starting distance Flight walking distance Flight walking speed
Schütz et al. (2012)	Raceway (15.0 × 1.5 m = 22.5 m^2)	1 step/s	Individual flight distance If calf can be touched
	Home pen (4.8 x 3.5 m = 16.8 m^2)	Not mentioned	Score between zero and four whether the observer could make eye contact (score 1), take one (score 2), or two steps towards the calf (score 3), or touch the calf (score 4) before it moved away, defined as moving both forelegs.
	Grass enclosure (15 × 3 m = 45 m^2)	Not mentioned	Individual flight distance
	Outdoor enclosure (9 × 10 m = 90 m^2)	Not mentioned	Score between zero and four whether the observer could make eye contact (score 1), take one (score 2), or two steps towards the calf (score 3), or touch the calf (score 4) before it moved away, defined as moving both forelegs.

2.3.2.3. Others

Other types of tests using humans were developed such as handling and stroking tests.

Firstly, the handling test was used by Frondelius et al. (2015) and Løvendahl et al. (2002). In the first paper mentioned, one objective was to assess whether dairy cows' emotional reactivity, as measured with their handling test, is connected to their heart rate variability values. The test was performed in experimental cubicles, where a female, previously unknown to the cows, handled them from both sides. The handling included touching and pinching of the animal, starting from the head and moving towards the rear of the cow. During those 6 minutes, an avoidance score based on the cow's behavioral response (score of 1 to 8) and the heart rate variability were measured. The handling test differs in the Løvendahl et al. (2002) experiment. In this case, animals were led by halter by personnel over a distance of 20 meters for cows and 50 meters for heifers. Only physiological measurements such as blood samples for concentrations of cortisol and adrenocorticotropic hormone (ACTH) were taken.

Secondly, Lange et al. (2020) and Proctor and Carder (2014) used a human stroking test. These experiments differ from the others since they aimed to investigate positive emotions. Stroking tests in both experiments were performed in 3 phases: pre-stroking, stroking and post-stroking. Each segment lasted 3 minutes in the case of Lange et al. (2020) and 5 minutes for Proctor and Carder (2014). A familiar stroker wearing rubber or canvas gloves applied a constant and previously practiced pressure while stroking at a frequency of 40-60 strokes/minute on lying animals. In Lange et al. (2020), stroking was directed to the ventral neck or was reactive, which is to say responding to perceived momentary preferences of the heifer. Both behavioral and physiological measurements were taken: they included postures (of the ears, head/neck, eyes, etc.) and heart rate. In Proctor and Carder (2014), the stroker concentrated their massage on the cow's neck, withers, forehead and cheeks. No physiological measurements were taken in this experiment. However, the scientists did look at ear postures and whether the cow was standing or lying.

2.3.3. Novel object test

The aim of the novel object test is to look at the fear of novelty (Boissy and Bouissou, 1995).

The environment where the test is performed varies from one article to the other and takes place in either a familiar or unfamiliar area. Arenas are mostly used. Kappel et al. (2017) used a race following the milking parlors. Schütz et al. (2012) conducted the novel object test in the home pen of calves housed in groups of five. As shown in Table 2.3.4, the object used in this test varied between the experiments (e.g. umbrella, balls, tambourine, etc.). Its position depends on the environment used in the test. When performed in the arena, the object is generally presented in the center of the arena. Although not mentioned by every article, some mentioned that the object was placed in order to be accessible to the animal (0.5 to 1.2 meters height). However, in the test performed in the home pen by Schütz et al. (2012), the object was dangling above the calves' heads, which is approximately 1.5 meters of height. To lower the object, Van Reenen et al. (2004) and Van Reenen et al. (2005) used a rope attached to the ceiling.

The animals can first be familiarized to the transport to the test area in order to facilitate handling. The distance ranges between 20 to 40 meters between home pen and arena. Different methods of transportation to the test pen are used depending on the experiment. Papers mention that the animal is either gently directed, herded, led by a halter or transported in a wheeled cart.

In general, the behavioral test is performed individually except in the case of Schütz et al. (2012) where the calves were in groups of five. The duration of the novel object test depends on the environment where it is performed. When done in an arena, the duration varies between 3 to 10 minutes of testing. In the case of Kappel et al. (2017), whom conducted the test in the pathway leading from the milking parlor, they ensured that each cow would stay for a maximum of 20 seconds in the area. The object is either already in the environment or introduced to the animal a few minutes after its entrance. This means that a human is probably not too far away to lower the object with a rope. In the experiment of Kappel et al. (2017), someone was present on the side of the race to record the cow's number and to ensure the animal would stay a maximum of 20 seconds. Also, during the test of Schütz et al. (2012), some observers were present outside of the calves' pen.

Measures. In the novel object test, behavioral measures are mostly taken. The latency to contact/approach and the duration of contact/interaction are the most popular measurements. Vocalizations and locomotion were also measured (see Table 2.3.4). Kappel et al. (2017) recordings are a bit different, since one of their objectives was to investigate lateralized behavior (i.e. behavioral expression of brain asymmetry). The observations are recorded during the whole time of the test. Only Van Reenen et al. (2005) and Van Reenen et al. (2013) took additional physiological measures. Blood samples for plasma cortisol concentrations were taken before and immediately after each test and heart rate was recorded during the novel object test.

In summary, the novel object test's objective is to observe neophobia by isolating an animal in an area with a novel object. Mostly behavioral measures, such as latency to contact/approach and duration of contact/interaction are recorded in order to investigate fear of novelty.

Table 2.3.4. Behavioral measures and description of the object reported in novel object tests

Reference	Novel object	Behavioral measures
Hedlund and Løvlie (2015)	- blue Pilates ball (diameter: 60 cm) - pink umbrella (diameter: 1 m) - blue and white plastic bag (60 × 60 × 25 cm)	Latency to approach Frequency of interaction Duration of interactions Duration standing within 2 m from the object
Kappel et al. (2017)	- two yellow semi-inflated party balloons (diameter: 12 cm) - two black/ white checkerboards (length 28 cm, width 20 cm, 4 x 4 cm squares) - light blue Kong™ (dog toy, diameter 7 cm)	Head orientation or eye use Physical object exploration Stop position
Lecorps et al. (2018)	black empty 50 L plastic bucket	Latency to contact Duration of contacts Time spent in proximity Total distance covered Number of vocalizations
Van Reenen et al. (2013)	tambourine connected to a blue plastic container (25 × 25 × 50 cm)	Latency to first contact Duration in contact Duration in locomotion Number of vocalizations
Van Reenen et al. (2005)	tambourine attached to a yellow plastic container (25 x 25 x 50 cm)	Latency to contact Duration in contact Duration in locomotion Number of vocalizations

		Number of defecations and urinations
Van Reenen et al. (2004)	tambourine attached to a colored plastic ball (diameter: 30 cm)	Latency to approach within 1 m Latency to contact Duration in contact Duration in locomotion Number of vocalizations
Schütz et al. (2012)	red rubber ball (diameter: 45 cm)	Latency to approach: 1) to touch 2) to stand within a calf length Duration of the interaction

2.3.4. Frustration/excitement test

Feed tests are often used to elicit frustration and excitement on cows. Conditioning and success vs fail tests are presented in this section.

2.3.4.1. Conditioning test

Lambert (Proctor) and Carder conducted a feed test using concentrates and woodchip (as a non-nutritional mock feed source) (Proctor and Carder, 2016; Lambert (Proctor) and Carder, 2017; Lambert and Carder, 2019). In their experiment, cows were brought individually into a familiar area. Those animals were used to being moved and were used to the presence of unfamiliar people. They were also conditioned to expect the delivery of standard feed when a bell was rung. In the first four trials, the animals were given standard feed to which they have continuous access to, thus creating a neutral stimulus. For the next five trials, cows were given concentrates, a feed that is highly desired and which they have limited access to (positive stimulus). In the last trial, cows were given inedible woodchip (negative stimulus). In these trials, 15-minute focal observations (pre-feeding for 5 minutes, feeding for 5 minutes and post-feeding for 5 minutes) were performed after a 10-minute period of acclimatization. In Lambert (Proctor) and Carder (2017) and Proctor and Carder (2016), only physiological measurements were taken. The first article examined the heart rate and eye whites. The second recorded the heart rate and nasal temperature. Finally, Lambert and Carder (2019) explored the effect of both positive and negative high arousal stimuli upon cows' ear postures and heart rate.

Agnethe-Irén Sandem described the behavior and the change in the eye whites of cows when expecting positive conditioned events (Sandem et al., 2006; Sandem et al., 2006). The stockman entering the area was regarded as the positive conditioned stimulus for feeding concentrate. The animals remained in their own stall and tests lasted 5 or 10 minutes. In both articles, the eye whites were observed during the test. Behavioral observations, such as head in or out of feed barriers, eating, head shaking, drinking, self-grooming, grooming the neighboring cow, vocalization, tongue rolling, and aggressive behavior towards neighboring cow were also examined during this period in Sandem et al. (2006).

2.3.4.2. Success vs fail

Another type of feed test has been used to elicit frustration and excitement. It is associated with feeding succeeded (pleasant) and feeding failed (frustration). In the two articles presented by Agnethe-Irén Sandem, the cows were first starved for 7 hours prior to the trial (Sandem et al., 2002; Sandem et al., 2006). In the three articles describing this test, they all used boxes or buckets with feed (Sandem et al., 2002; Sandem et al., 2006; Lv et al., 2018). In the positive test, the animals have access to the feed in the box or bucket. In the negative test, the animals are introduced to the box or bucket full of feed, but it has a cover with holes allowing to both see and smell, but not to reach the feed. Time of test (or exposition to stimulus) was of 6 and 10 minutes for Sandem et al. (2002) and Sandem et al. (2006) respectively. In the case of Lv et al. (2018), animals were exposed for 10 minutes to the positive stimulus and 40 minutes to the negative stimulus. Eye whites were only observed in Sandem et al. (2006) during the 10-minute test. Additionally, Sandem et al. (2002) looked at the frequencies of vocalization, tongue rolling, aggressive behavior and head shaking during the 6-minute test. Lv et al. (2018) measured heart rate and took samples of saliva and blood for immune parameters for a 20-minute period before positive and negative test periods. Also, heart rate was taken again during the last 5 minutes of the positive test. Saliva and blood samples were then collected following the positive test. Afterwards, Lv et al. (2018) took behavioral measurements looking at self-grooming, exploration, locomotive play, head shaking and tongue rolling. We can assume that the same procedure was used with the negative test.

2.3.5. Emotions tested in arena, human, novel object and frustration/excitement tests

In sum, as presented in Table 2.3.5, the behavioral tests discussed in this review are mostly examining fear, the few other iterations of tests examine frustration (another negative emotion) or positive emotions.

Table 2.3.5. Summary of emotions tested in tests in the present scoping review (n = 26 articles)[1]

	Fear	Frustration	Positive
Arena test	✓(n = 5)		
Human stationary test	✓(n = 6)		
Human approach test	✓(n = 7)		
Handling test	✓(n = 2)		
Stroking test			✓(n = 2)
Novel object test	✓(n = 7)		
Feed test		✓(n = 6)	✓(n = 8)

[1]Note: One article can appear in more than one line.

2.4. DISCUSSION

In this section, factors to consider when planning arena, human, novel object and frustration/excitement tests are discussed. Limits associated with them are also presented.

2.4.1. Arena test

When planning the arena test, many elements should be considered carefully. First, the arena has to be different from the home environment to make it novel (for example, in terms of dimension, construction of walls or floor, bedding or location, whether indoor or outdoor, and how stimulating the novel environment is). The location of the arena has a great influence. For example, for a cow always kept inside, an outdoor arena may be associated with novelty (noises, wind, temperature, view, odors, floor, weather, etc.). Another factor to plan is the transport of the animal to the test area. It should be done with minimal stress and using the same manner with all subjects in order not to influence the reaction of the animal on the later test. In fact, one of the most important things is to transport the animal gently and calmly, as mentioned in most of the articles reviewed. There should be minimal contact between the cow and the human before the test in order to reduce the influence of the animal's fear of humans. Similar considerations should be taken when placing humans around the open field. Since the focus of this test is the fear of novelty and not the fear of humans, persons should not be around the novel environment and the animal. Kilgour (1975) does not mention if the observers in the observation towers could be seen by the cows, which would have been crucial. Finally, the test should be performed at the same time of day when repeated over time. Otherwise, the animal could act differently because of its general activity varying throughout the day.

Different limits are associated with this test. First of all, when thinking about replicating the novel arena test over time, it is challenging to keep this environment novel to the cow for each replicate of the test. Indeed, as experienced by Kilgour (1975) who brought cows into the same arena three times on three consecutive days, the animals showed a clear decline in both ambulatory and vocalization scores over the course of the experiment. They reached the conclusion that the test area might not have stayed novel for much more than one day. Secondly, a problem is encountered when the social animal is isolated from the rest of the herd during testing (Kilgour, 1975). The fear of isolation could affect the interpretation of the fear of novelty. Finally, one factor which should be considered when interpreting results is the curiosity of the animal (Van Reenen et al., 2004). The exploratory tendency could mediate the reactivity to this kind of alarming situation. Articles using the arena test need to consider these points in their interpretation to caution their explanation of the reasons why the animal is or isn't reactive to the test.

2.4.2. Human test

2.4.2.1. Human stationary

Different elements should be considered when planning a human stationary test. It is important to transport the animal to the test area with minimal stress and, if possible, train it prior to the test. The stress resulting from the transport and handling could influence the reaction of the animal during the human stationary test. The same thing should be considered when using observers. Furthermore, the environment where the test is performed should not be novel, the interest being on focusing on the fear of humans and not on the reaction to a novel environment. The human involved can be familiar or unfamiliar. However, the animal having a previous experience with a person can influence the results in a positive or negative way. Choice of human (familiar or unfamiliar) impacts the research question asked. Since the animal can discriminate between handlers wearing overalls of different colors (for example), clothes (color and probably style) should be different from what the cow or the calf usually sees (handlers, veterinarians, etc.) (Rushen et al., 1998). Not only clothes, but human stature, odors and other factors might also need to be considered. If possible, the presence of observers in full view of the animal should also be avoided in order to reduce its impact on the observations. As mentioned for the arena test, the human stationary test should be performed at the same time of day when repeated over time.

Even if all these elements are considered carefully, some challenges remain. When repeated over time with the same person, there is a risk of habituation. There is also the risk that behavioral responses in the voluntary approach test reflect other motivations such as exploration (Sutherland and Huddart, 2012).

2.4.2.2. Human approach

When planning this test, some elements should be considered. As mentioned for the human stationary test, stress should be minimal during transport. As done by Sutherland and Huddart (2012), it is possible to always use the same handler during the experiment. The use of an observer should also be considered with caution. For the same reasons mentioned in the previous test, the environment should be familiar to the animal. The decision to use an unfamiliar or a familiar human, the choice of the clothes all depends on the specific goals of the project. In the human approach test, it is essential that the human practices before testing in order to keep the same method during testing (same speed and posture). Finally, the human approach test should be performed at the same time of day when repeated over time.

The limit of the human approach test is related to the possible habituation of the animal when the test is repeated over time. The animal becomes more and more familiar with the person. However, Sutherland et al. (2012) add that the human approach test may more accurately assess fear of humans than the human stationary test, since the latter may assess other motivations such as curiosity.

2.4.2.3. Others

Handling. Frondelius et al. (2015) mention that their test (touching and pinching of the animal for 6 minutes) might not have been a sufficiently negative stressor. The authors added that previous positive experiences can affect animals' reactions in later human-cow interactions and that in their case, cows were already used to handling situations. In order to have an appropriate reaction to the human, the animal should not have any previous experience with the person performing the test. Researchers who want to perform handling tests should also be careful when repeating them over time in order to avoid habituation and influence fear reaction. Additionally, the handling test should be performed at the same time of day.

Stroking. These experiments are different from the others since they aim to investigate positive emotions. The main condition for the success of the stroking test is the cow being positively habituated to the procedure, equipment and researchers. However, this test is still intrusive and could easily be associated with fear responses. As mentioned for the handling test, the stroking test should be performed at the same time of day when repeated over time.

2.4.3. Novel object test

When planning the novel object test, different factors should be considered. As mentioned for the arena test, transport to the test area should be done with minimal stress in order to reduce the influence on the subsequent test. As mentioned by Hedlund and Løvlie (2015), the novel object test should be performed in a familiar environment in order to avoid reactions related to a novel area. Otherwise, if it has to occur in an unfamiliar environment, giving a few minutes to allow familiarization might help. Subsequently, the object should be different (novel) from what the animal is used to see in its environment. Humans around the experimental area should be avoided, especially if they can be seen by the animal, as this could influence its response (fear of humans). Finally, the novel object test should be performed at the same time of day when repeated over time.

This test has different limits. First, one challenge is to keep the object novel. Repetition of the same test with the same object could be associated to habituation and would no longer produce a fearful response from the animal (Hedlund and Løvlie, 2015). Kappel et al. (2017), for example, might have observed learning effects or habituation by presenting the same object on three consecutive days. This is the reason why a new object was used for each observation in Hedlund and Løvlie (2015). Secondly, the test might not be able to show neophobia, but rather the curiosity of the animal (Schütz et al., 2012).

2.4.4. Frustration/excitement test

2.4.4.1. Conditioning test

When planning this type of test, researchers should make sure the animal is well-conditioned (in the case of the article presented, the animal would expect the delivery of standard feed when a bell was rung) and habituated to the set-up in order to get the appropriate reaction. Lambert (Proctor) and Carder (2017) mentioned that they were not sure the cows were truly conditioned, so it would have been interesting to conduct more standard feed and concentrate trials. In Sandem et al. (2006) case, they ensured that the animals were conditioned since the feeding routines in the stall had been the same for years. Also, the presence of humans during tests should be avoided or the animal should be used to unfamiliar people. For example, in Lambert (Proctor) and Carder (2017), it would have been beneficial to use an automated device instead of a human to ring the bell. If this kind of test is repeated, it should always be done at the same time of day.

2.4.4.2. Success vs fail

No limit associated with this test was mentioned by the articles. However, someone considering reproducing this feed test should remember to habituate the animal to the environment and the experimental set-up in order to elicit the right emotion, in this case frustration or pleasant emotion. Experimenters should also be careful when repeating this test and should perform it at the same time of day.

2.5. FUTURE PERSPECTIVES AND CONCLUSION

Emotional tests on cattle presented in this scoping review are complex and many details need to be carefully controlled, both in their performance and interpretation. Thus, the applicability in farms in order to evaluate cow welfare could remain problematic (time consuming, staff needed, not automated, etc.). Even if some tests are used in routine, such as the human test due to being less time-consuming and requiring less staff, there is a need for more practical means in order to facilitate uptake. Simpler tests or measures (such as a simplified version of the human test as used in Welfare Quality® protocol (Welfare Quality®, 2009)) or remote or indirect evaluation of emotions using a combination of indicators instead of performing a test might be helpful in the future. As mentioned by Neethirajan et al. (2021) there is still limited application of the sensors available to observe the emotions of farm animals. Additionally, few tests had both behavioral and physiological measurements, which should be considered in the future. Most of the reviewed tests were performed on negative emotions and there seems to be a lack of available tests on positive emotions, but there is growth of those looking at mood, with tests such as judgment bias (Lecorps et al., 2019). Also, with the tests presented here, there is still a possibility to observe positive emotions. It might be possible to examine positive emotions with tests, but researchers would need to go further in their interpretation in order to do so. Research should be done on positive emotions in order to have a global view of the state of animals. The promotion of positive emotions, not only the removal of negative ones, would help in reaching a net positive welfare state (Proctor and Carder, 2014).

In conclusion, even if there is a growing interest for animal welfare and cows' emotions, available emotional tests are complex and can be hard to apply and interpret, and therefore limit their uptake for on-farm animal welfare evaluation. Most of the articles used in this review used tests designed to look at fear. Thus, in order to improve cows' welfare to a net positive welfare state, other tests should be used or refined in order to broaden the range of emotions measured, and particularly to measure positive emotions in cattle.

2.6. GENERAL AND SPECIFIC OBJECTIVES

2.6.1. General objectives

The general objective of this thesis is to review the behavioral tests currently used to examine bovines' emotions in existing literature and to determine the influence of indoor and outdoor exercise access on the motivation and reactivity of cows housed in tie-stalls.

2.6.2. Specific objectives

The specific objectives of the scoping review included in this thesis are to:

1. Describe various aspects of behavioral tests: aim (and specifically: which emotion is targeted), environment where is performed the test, conditions before and during the test, and measurements.
2. Describe important factors to consider when planning a behavioral test.
3. Describe limits of behavioral tests.

More specifically, objectives of the experimental study in this thesis are to:

1. Determine the motivation of the cow to go to an indoor or outdoor exercise area. We hypothesized that in both types of exercise areas, cows would be motivated (increase of trip and total speed over time), but a higher motivation of the cows to go to outdoor exercise area would be observed because of a more enriched environment.
2. Determine the reactivity of the cow based on its treatment (experience in the indoor or outdoor exercise area during the first period of application of the treatment). We hypothesized an improvement of indoor and outdoor cows' reactivity to humans (decrease of latency and increase of scores) because of the more positive handlings by them. We hypothesized the same improvement for the cows' reactivity to suddenness (reaction closer to score 1 and freezing time decreasing) and novelty in being more confronted to these kinds of events. An improvement of the cows' reactivity to novelty could be shown, notably by a decrease of fear reaction such as a decreased distance between object and cows. This could possibly improve curiosity and manifest itself through an increased frequency of interactions e.g., licking.

3. Determine the reactivity of the cow in relation to the overall experience (before and after the two periods of treatment application). We hypothesized the same time effect as objective 2, perhaps with more intensity.

CHAPTER 3 – THE EMOTIONAL RESPONSES OF TIE-STALL COWS TO INDOOR AND OUTDOOR EXERCISE ACCESS

3.1. MATERIAL AND METHODS

3.1.1. Ethics statement

The use of animals and all procedures were approved by the Animal Care Committee of CRSAD (Deschambault Animal Science Research Centre) (protocol #1920-BL-387) and by the Animal Care Committee of McGill University and affiliated hospitals and research institutes (protocol #2016-7794).

3.1.2. Animals and management

Twenty-seven dairy cows were selected from the Dairy Facility of the CRSAD (Deschambault, Quebec, Canada) for this experiment. It was conducted between January and March of 2020. The cows were housed in tie-stalls of 3.3 m^2 (1.8 m x 1.5 m + 0.4 m x 1.5 m) on a concrete floor covered with a rubber mat. Straw was used as the bedding. The animals were grouped in 9 blocks by parity and days in milk (respectively, Parity: 2.22 ± 0.93; DIM: 161 ± 67) (average $\pm$ SD). They had free access to water and were fed a total mixed ration twice a day at 8:30 AM and 1:15 PM. Furthermore, the cows were milked twice a day, at 7:00 AM and 4:30 PM.

3.1.3. Experimental treatments

Eight days were used to habituate all cows to procedures and exercise areas. It was also an occasion for the handlers to get used to the procedures. During this period, all cows were randomly assigned to a treatment (outside, inside, stall) for each day so that each animal went over each treatment by the end of the habituation period. The time spent per cow in the exercise areas was increased over time, starting at 5 minutes on days 1 and 2, 15 minutes for days 3 to 5, and 25 minutes from days 6 to 8.

Initially, the experiment consisted of three periods of three weeks with a wash-out period of five days. However, because of the COVID-19 pandemic, the last period had to be abandoned.

Throughout the experiment, cows were continuously housed in the same stall when not undergoing the exercise treatments. The animals were randomly allocated to 1 of 3 treatments per period (9 animals per treatment). **Treatment 1 (S):** No exercise access. The animal stays in its own stall continuously and has access to water and feed (Figure 3.1.1). **Treatment 2 (I):** Indoor exercise access. The animal goes into an indoor exercise area for 1 hour a day for 2 consecutive days a week. The pen is 29.60 m^2 (7.29 m x 4.06 m). It is made of three walls and one gate allowing cows to see other cows in their stall and a concrete floor covered with wood shavings. The cows did not have access to water or feed during the treatment. The area is located close to the feeding room and the calf's area (Figure 3.1.1). To prevent any distraction related to them, a tarpaulin is installed to avoid visual contact with calves. However, the animal in the indoor exercise area could see other cows in their stall (stalls that are in front of the area). No one was allowed to stay voluntarily in front of the pen during treatment application in order not to disturb the animal. At the end of the day, dirty wood shavings contaminated by manure or urine were removed from the pen. **Treatment 3 (O):** Outdoor exercise access. The cow goes into an outdoor exercise area for 1 hour a day for 2 consecutive days a week. Each animal was placed outside in one of the two individual exercise areas (Figure 3.1.1). The area used by the treatment cow was randomly allocated each day during the whole study. The treatment cow would always be paired with one companion animal to avoid social isolation. The companion cow was attributed to the second outdoor exercise area. The companions were two primiparous cows. One of them was allocated to the two outings in the morning and the other one was allocated to the outing of the afternoon. The pens are 28.73 m^2 (5.36 m x 5.36 m) and made of gates sustained by beams of wood. The exercise areas are constructed on biofilms (as part of an environmental project done at the same time) covered by cedar mulch. Since the experiment was conducted during winter, snow covered the outdoor areas most of the time. The cows didn't have access to water or feed during the treatment application. Disturbance of the animals undergoing outdoor exercise treatment was minimal as no people and equipment were permitted to circulate close to the areas during treatment application. At the end of the day and during the entire experience, the manure was never removed from the pens, because engineers were taking measures related to it. Instead, manure was spread around the areas for environmental research. The maintenance of the outdoor alley leading animals to the exercise areas followed a specific standard operating procedure (Supplemental Text 1).

Treatment cows were allocated to a single one-hour period of outing per day. Three time slots were available: the first outing of the day (AM1, around 9:20 AM), the second (AM2, around 10:50 AM) or the third one (PM, around 1:50 PM). Treatment cows were allocated to two consecutive days of outings per week either Monday-Tuesday (D1-D2), Wednesday-Thursday (D3-D4) or Friday-Saturday (D5-D6). The allocation of the moment of the outing of the day and the days of outing were the same for each cow during the whole experiment.

To decide if a cow was able to undergo its treatment application (inside or outside), we first determined if the cow was in heat. Also, for the outdoor treatment, we determined if conditions (temperature, rain, snow and ice) were appropriate and, if not, what could be done to improve them (Supplemental Text 1 and Supplemental Table 1). Those decisions were taken at the first outing of the day and these steps were followed again if there was a change in conditions (e.g., a storm starting at noon). When it was decided that cows could undergo their treatment application, the handler and the experimenter would start to prepare for the outing around 15 minutes before the scheduled outing. A protocol was developed to specify the order of the steps to follow (Supplemental Text 2). After preparing the outing, the handler would first bring the companion cow outside (only for AM1 and PM, because the same companion cow from AM1 would stay outside for the outdoor cow of AM2). Then, the cow attributed to the outdoor treatment would be brought to the second outdoor exercise area (see Figure 3.1.1). The outdoor area associated with a treatment cow could change from one day to another because it was determined randomly. Finally, the handler would bring the cow attributed to the indoor treatment to its exercise area (see Figure 3.1.1). Average distance to outdoor and indoor areas were 34.0 m (min= 21.7 m (left area), max= 51.2 m (right area)) and 18.6 m (min= 3.6 m, max= 28.6 m), respectively. After one hour of treatment application, the handler would bring back the cows to their stall in this order: 1- outdoor treatment cow, 2- indoor treatment cow, and 3- companion cow.

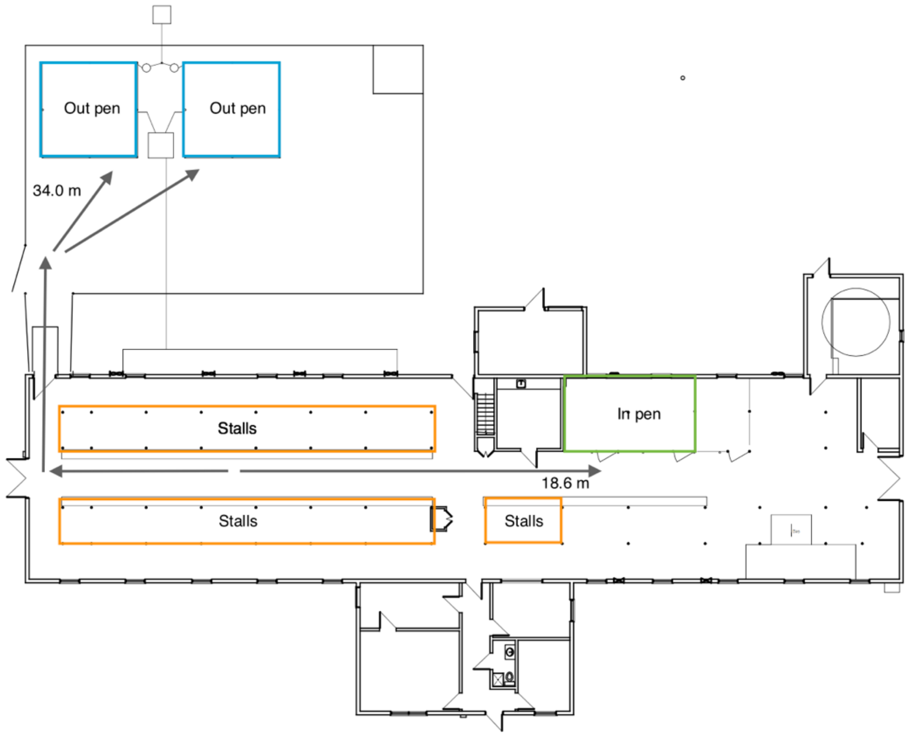

Figure 3.1.1. Map of the barn with stalls, indoor and outdoor exercise areas. Distance to indoor area: avg= 18.6 m, min= 3.6 m, max= 28.6 m. Distance to outdoor areas: avg= 34.0 m, min= 21.7 m (left area), max= 51.2 m (right area).

Three trained handlers led the cows during the trial following a specific protocol for walking the cows (Supplemental Text 3). However, only two of them (A and B) were part of the trip test described later. One of them was part of the trip test for 90 % of time and the second for 10 % of the time.

3.1.4. Behavioral tests

Three tests were performed in order to look at the various components of the reactivity of the cows: the human test, the suddenness test and the novel object test (Boissy, 1995). The procedure of the human and suddenness tests is adapted on what had been done previously by Aigueperse and Vasseur (2021), while the novel object test was adapted from Herskin et al. (2004). Depending on the outing day (D1-D2 or D3-D4 or D5-D6), nine cows received all behavioral tests in one day. A total of three days was necessary to perform tests on the twenty-seven dairy cows. An example of one testing day is presented in the Supplemental Table 3. The tests were performed at three different times of the day: during first outing (around 9:45 AM for session 1), over lunchtime (around 12:15 PM for session 2) and after the last outing of the day (around 3:15 PM for session 3). Due to the lack of time to conduct all tests during session 1 and 2, the suddenness test for these two sessions was performed at 11:30 AM (during the second outing), and at 2:15 PM (during the third outing). In each session, the human test was the first one for all cows and was performed once per cow, three times per day. The results of the sessions were later averaged. The novel object and the suddenness test were performed once per cow per day. The suddenness test was always the third test performed in each session, because this one is more likely to disturb the cows. The order of the cows subjected to tests in a session depended on their placement in the barn, in order to prevent the focal cow from being disturbed before behavioral tests. Also, for all behavioral tests, two neighbors were never tested at the same time or right after the other one.

The choice of the moments when to perform the tests was based on different criteria. First, no one was allowed to circulate close to the focal cows for the disturbance of the animals to remain minimal. For the same reason, tests were never performed while a cow was being led to its exercise area, during feeding, milking, maintenance of the stalls or over any other moments of interaction with cows.

The human and suddenness tests were performed four times over the trial for all cows: before and after the habituation period, after periods 1 and 2. The novel object test was performed three times only: before habituation, after period 1, and after period 2.

Heat or any exceptional event that could disturb the animal the day of the test was written down.

All suddenness and novel object tests were recorded onto videotape using tripod cameras. The same cameras were used for both: GoPro Hero5 Black and GoPro Hero7 Black (GoPro inc., San Mateo, CA, USA). For those tests, the camera was placed on the ground with a tripod in front of the cow in its tie-stall (a little less than 2 meters in front of the animal). The animal could get used to this material for a period of 1 minute 30 seconds and 5 minutes respectively before the beginning of the suddenness and novel object tests.

3.1.4.1. Human test

The aim of the human test is to observe the reaction of the animal to an approaching human in its home environment (Aigueperse and Vasseur, 2021). To do so, we used an unknown human to the cows (someone who had never and will not set foot on the barn). We used the same person during the whole experiment. It was a woman who always wore a white plastic overall, her head covered with a hood, and black rubber boots. This person was trained before the first test. The observer (or experimenter) was also always the same person wearing blue overalls. This person was trained by someone used to this procedure and practiced by observing cows in a different barn.

Two minutes prior to the tests, the observer would gently ask the focal cows to stand. If the cow decided to lie down between this time and the test, the observer would ask the cow to stand once again. Then, the human stimulus would go two meters in front of the cow in her tie-stall to start the test.

Afterwards, the test was performed for 25 seconds in five stages (five seconds per stage):

Stage 1: human stands in front of the cow in its tie-stall, his arms placed alongside the body.

Stage 2: one step closer to the cow, arms placed alongside the body.

Stage 3: one step closer to the cow, arms placed alongside the body.

Stage 4: one arm stretched out at approximately 45 degrees.

Stage 5: with the arm stretched out, the human moves forward and tries to catch the chain at the base of the neck.

Over this period of time, the observer was two stalls away from the focal cow and the human and scored the reaction of the animal at each stage following the scoring reported in Table 3.1.1.

Table 3.1.1. Scores used in the human test[1]

-3	-2	-1	0	+1	+2	+3
Steps back, chain stretched to the maximum OR in diagonal backwards (AND/OR head hidden)	Steps back / struggles (for stage 5 only)	Turns the head back or away	Looks at the person	Approaches the head without touching, sniffs	Approaches and touches the person	Tries to lick / catch the person with the mouth (clothing or hand with the tongue or the lips)

[1] Adapted from Aigueperse and Vasseur (2021).

The observer also wrote down the latency to touch the human (time when the cow first touches the person voluntarily).

This test was repeated three times per cow for each test day, once per session. Those results were later combined to get a mean reaction per stage of the three tests done in one day.

3.1.4.2. Suddenness test

The aim of the suddenness test is to observe the reaction of the cow facing a sudden event: the quick fall of a white plastic bowling pin (H= 45 ∅= 10 cm) in front of the cow in her tie-stall (Aigueperse and Vasseur, 2021). In order to do this, the object was first installed at least twelve hours prior to the test. The bowling pin was attached to the ceiling with a string, in a way that it could not be seen by the animal and where it can fall in front of the cow in the feeding area. The string attached to the pin was tied at least two stalls away from the focal cow in order to reduce the disturbance by humans at the moment of the test.

Two minutes prior to the test, the observer would gently ask the focal cow and the two neighbors to stand. Then, panels were placed in the gutter behind them. The cow was first video recorded for 1 minute 30 seconds, then the experimenter would use the string to quickly drop the bowling pin in front of the animal in the feeding area. The cow was recorded for another 30 seconds after the fall. The recordings were later used to observe freezing time by taking the minimum between the latency to touch the pin and latency to regain its behavior. The reaction at the fall was also noted based on the scoring shown at Table 3.1.2.

Table 3.1.2. Scores of reaction used in the suddenness test[1]

0	1	2	3	4
No reaction	Startled, with no backward movement	Startled, with backward movement of 1 step	Startled, with backward movement of more than 1 step	Startled, with backward movement of more than 1 step (with struggle), taut chain OR in diagonal backwards (AND/OR head hidden)

[1] Adapted from Aigueperse and Vasseur (2021).

For both the human and suddenness tests, scores were considering the fact that cows could not go backwards as much as they could have because of the opened gutter. Thus, compared to the original article of Aigueperse and Vasseur (2021), we added the options of diagonal backwards and/or head hidden.

3.1.4.3. Novel object test

The aim of the novel object test is to observe the reaction of the animal to a new object placed in its familiar environment (tie-stall) (Herskin et al., 2004). The object used is a ball of 38 cm of diameter and of different colors (blue, green and pink) and patterns (filled, white striped, white stippling). The smooth texture was identical for all of them. The balls were different every time a cow would be tested in order to make the object as novel as possible without changing its shape and its usefulness to the animal. As for the suddenness test, the object was installed at least twelve hours beforehand. Moreover, it was installed in the same way and at the same place as the bowling pin.

To perform the test, the two neighbors were gently asked to stand in order to be able to shorten their chain and to prevent them from interacting with the object of the focal cow. Some minutes after chain shortening, the focal cow was then recorded for 5 minutes. Then, the experimenter would use the string to slowly drop the ball in front of the cow until it touches the ground. The animal was recorded another 5 minutes after the fall.

For the analysis, a trained observer used the recording to look at the frequency and duration of all the behaviors observed before and after the fall and the distances (Table 3.1.3 and

3.1.4). The latency was also observed for the behaviors related to the novel object test (Table 3.1.4) except for the behavior of looking at the object.

Results associated to behaviors observed before novel object test will only be presented in Appendix Table 1 and 2.

Table 3.1.3. Ethogram of behaviors observed before novel object test[1]

Observation type	Behavior	Description
Resting		No activity, including sleeping and ruminating, for at least 10 seconds without being alert or anxious
Ruminating		Without being alert: regurgitation, chewing and swallowing of previously eaten food (animal may be lying or standing)[2]
Ingestion	Eating	Searching, grabbing, chewing and swallowing feed
	Drinking	The muzzle is located in the water bowl, with ingestion of fluids[3]
Grooming		Licking or rubbing own head or body surface with self or infrastructure[3]
Elimination	Defecating	Elimination of feces from the body[3]
	Urinating	Elimination of urine from the body[3]
Social interactions	Fighting	Head butting with strength, fighting, forcing with one part of the body against another cow[4]
	Receiving fighting	Another cow is head butting with strength, fighting, forcing with one part of the body against the focal cow. All behavior of the focal cow associated with receiving fighting.[4]
	Social grooming	Licking the head, neck, or body of the other cow (tongue in contact with the fur)[5]
	Sniffing other cow	Muzzle in contact with the companion or within one muzzle-width of the companion[5]
Exploration	Sniffing environment	Sniffing ground, feed or parts of her stall
Postures	Standing	A cow was classified as standing when all 4 limbs were fully extended and perpendicular to the ground [6]
	Lying	Cattle were considered lying when the flank of the animal was in contact with a surface [6]

[1] It was decided to analyze the four minutes before the fall. Also, all the behaviors lasting one second or less were considered as frequencies and no duration were written down. The duration of a behavior was considered "done" when the animal stops exhibiting it for at least five seconds or more, or starts displaying another behavior. Activity of the cow (active or inactive) was also investigated. Active was defined as the cow engaging in ingestion, social interaction or in exploration. Inactive was defined as the cow resting or grooming.
[2] Adapted from Kerr and Wood-Gush (1987).
[3] Zambelis et al. (2019).

[4] Adapted from Fukasawa and Tsukada (2010).
[5] Adapted from Duve and Jensen (2012).
[6] Adapted from Borchers et al. (2016).

Table 3.1.4. Ethogram of behaviors observed during novel object test[1]

Observation type	Behavior	Description
Behaviors related to novel object	Look	Eyes are directed towards ball
	Approach	Cow approaches the ball with interest or alert toward the object
	Move away	Cow moves away from the ball with interest or alert toward the object
	Sniff	Muzzle close or in contact with the ball
	Lick	Tongue in contact with the ball
	Push	Head in contact with the ball to push or roll
Behaviors not related to novel object	Resting	No activity, including sleeping and ruminating for at least 10 seconds without being alert or anxious
	Ruminating	Without being alert: regurgitation, chewing and swallowing of previously eaten food (animal may be lying or standing)[2]
	Eating	Searching, grabbing, chewing and swallowing feed
	Drinking	The muzzle is located in the water bowl, with ingestion of fluids[3]
Distances	Touch	Cow touches the ball voluntarily or not
	< 1 head	Less than one head of distance between the cow and the ball
	1 to 2 heads	Between one and two heads of distance between the cow and the ball
	> 2 heads	More than two heads of distance between the cow and the ball
	Stretched maximum	The furthest the cow can get from the ball

[1] It was decided to analyze the whole five minutes following the fall. Also, all the behaviors lasting one second or less were considered as frequencies and no duration was written down. The duration of a behavior was considered "done" when the animal stops exhibiting it for at least five seconds or more, or starts displaying another behavior.
[2] Adapted from Kerr and Wood-Gush (1987).
[3] Zambelis et al. (2019).

3.1.5. Trip scoring

The aim of this observation is to test the motivation of the animal for the outing either in the inside or outside exercise area. In order to do this, following the SOP for leading the cows to their exercise area (Supplemental Text 3), a trained handler would put on a halter on the cow in its tie-stall and use it to lead it to its attributed treatment exercise area (inside or outside). An observer would make live observations of the whole process from the start (the installation of the halter), five meters behind the cow with a stopwatch. This person would make an intervention only in the case where the cow is not moving forward (Supplemental Text 3).

Observations were performed four times, which is to say at the first and third week of periods 1 and 2. Some measures were also taken on the first week of period 3.

The observer measured the time needed to put on the halter, which started when the handler would first pass the cow's shoulder and raise its arms trying to first put on the halter on cow's head. This measure ended when the animal had four hooves in the alley. The duration of the trip, from the stall to the exercise area, was also measured and is described as the time from the moment the cow has four hooves in the alley until the latter are in the exercise area. These two durations were combined in order to measure the total duration of the manipulation, which becomes the time from the moment we start to put on the halter until the cow has four hooves in the exercise area.

We also measured the distance from each stall to each exercise area for each cow. These measures with the durations allowed us to calculate the trip speed (distance / duration of the trip) and the total speed of manipulation (distance / total duration) (m/s).

3.1.6. Animal care monitoring

Four measures of animal care monitoring were taken at regular intervals during the whole experiment by a trained individual; body condition score (following: Vasseur et al. (2013)); body injuries (St John et al. (2021)); cow cleanliness (Vasseur et al. (2015)) and lameness (Palacio et al. (2017)). Those four measures of welfare were taken at four different times during the experiment: before and after habituation and after periods 1 and 2. Cleanliness was also evaluated after the second outing of the week for each cow for weeks 1 and 3 of each period in order to specifically monitor cow cleanliness after an outing.

These measures were taken for the sole purpose of animal care monitoring and are not reported or a subject of analysis in the thesis.

3.1.7. Video analysis and repeatability

VLC (Version 3.0.7.1 Vetinari (Intel 64bit)) was used to analyze the recordings of suddenness and novel object tests. For both tests, observer's reliability was assessed by comparing the observer scores with an expert's. This trained observer then analyzed all the recordings from those two behavioral tests. Over this procedure, the observer was blind to the treatment. In the case of the novel object test, intra-reliability was performed the whole time.

Overall average of intra-observer reliability is 92 % for the behaviors and 79 % for the distances. This average was computed with the percentage of observations with more than 80 % of agreement. More information on repeatability and detailed results are reported in the Supplemental material section (Supplemental Table 2).

3.1.8. Statistical analysis

3.1.8.1. Data handling

Data handling was necessary in the case of the novel object test. Frequencies for behaviors and distances were divided by duration of observation, which was normally 240 seconds before test and 300 seconds during test. They were then multiplied by 60 in order to get frequencies by minutes. Latency was computed as the difference between the moment the first behavior occurred and the beginning of the test. In order to compute durations, different categories were developed to account for the fact that some behaviors could be performed simultaneously. Three main categories were developed for pre-test data: ingestion, social and exploration. Postures, rumination and receiving behavior were analyzed apart. For data related to the novel object (during test), looking was calculated apart from interaction behaviors. Rumination was calculated apart from the rest of the behaviors not related to the novel object. Percentages of observations for each of the behaviors and of the behavior categories were obtained by dividing their durations by the total duration of the observations (typically 240 seconds before the test; 300 seconds during the test). These percentages were computed to look at the time spent before or during test on a behavior or a category. In order to compute the percentage of a behavior within a category, the duration of this behavior was divided by the duration of the category. This percentage was obtained to show proportions within one category. Additionally, only behaviors which occurred for more than one second were accounted for in the durations. Finally, defecation, urination, approach and moving away were not computed for duration, since those behaviors were considered as events.

3.1.8.2. SAS

The initial experimental design was a repeated 3x3 Latin square. Even though one period had to be abandoned because of the COVID-19 pandemic, we kept this design for the trip test model and considered period 3 as missing data. For objective 3, period 3 was taken out of the dataset altogether. All statistical analyses were performed using SAS 9.4 (SAS Institute Inc., 2012, Cary, NC, USA).

Objective 1 - Determine the motivation of the cow to go to the indoor or outdoor exercise area (trip test)

One model was developed for the analysis of variables from the trip scoring:

$$Y_{ijklmn} = \mu + period_i + week(period)_{ij} + trt_k + week(period)*trt_{ijk} + block_l + cow(block)_{lm} + person_n + e_{ijklmn}$$

Where: Y_{ijklmn} is the dependent variable, which is the outcome measure of the m^{th} cow of the l^{t} block during the k^{th} treatment during the j^{th} week and the i^{th} period with n^{th} person. The $period_i$ is the fixed effect of the i^{th} period (i= 1 to 3). The $week(period)_{ij}$ is the fixed effect of the j^{th} week (j= 1 and 3) from the i^{th} period. The trt_k is the fixed effect of the k^{th} treatment (k= inside, outside). The $week(period)*trt_{ijk}$ is the fixed effect of the interaction between the k^{th} treatment and the j^{th} week from the i^{th} period. The $block_l$ is the fixed effect of the l^{th} block (l= 1 to 9). The $cow(block)_{lm}$ is the random effect of the m^{th} cow (m= 1 to 3) within the l^{th} block. Finally, $person_n$ is the fixed effect of the n^{th} person manipulating the cow (n= A, B) and e_{ijklmn} is the random error.

To account for the repeated measures, the best fitting covariance structure (among Auto-Regressive (1) or AR1, Compound Symmetry or CS, and Unstructured or UN) was selected for each outcome measure following the BIC fit statistics assessment. Normality of residuals for each outcome measure was tested using PROC MIXED and PROC UNIVARIATE procedures.

A Bonferroni adjustment was used in order to account for the multiple comparisons associated with the interaction between week(period) and treatment.

Objective 2 – Determine the reactivity of the cow in relation to its treatment (experience in the indoor or outdoor exercise area during period 1 only)

Data from before and after period 1 were analyzed for this objective. One model was developed for the analysis of variables from the human test, the suddenness test and the novel object test, with regard to the effect of each treatment:

$$Y_{ijkl} = \mu + block_i + trt_j + cow(block\ trt)_{ijk} + time_l + trt{*}time_{jl} + e_{ijkl}$$

Where: Y_{ijkl} is the dependent variable, which is the outcome measure of the k^{th} cow of the i^{th} block during the j^{th} treatment at l^{th} time. The $block_i$ is the fixed effect of the i^{th} block (i= 1 to 9). The trt_j is the fixed effect of the j^{th} treatment (j= inside, outside, stall). The cow(block trt)$_{ijk}$ is the random effect of the k^{th} cow (k= 1) within the i^{th} block. $Time_l$ is the fixed effect of l^{th} time (l= before, after). The trt*time$_{jl}$ is the fixed effect of the interaction between the j^{th} treatment and the l^{th} time. Finally, e_{ijkl} is the random error.

To account for the repeated measures, the best fitting covariance structure (among Auto-Regressive (1) or AR1, Compound Symmetry or CS, and Unstructured or UN) was selected for each outcome measure following the BIC fit statistics assessment. Normality of residuals for each outcome measure was tested using PROC MIXED and PROC UNIVARIATE procedures. A Bonferroni adjustment was used in order to account for the multiple comparisons associated with treatments and interactions between treatments and times. Also, estimate statements were used to compare treatment effect (inside and outside) with stall.

Objective 3 – Determine the reactivity of the cow in relation to the overall experience (periods 1 and 2)

Data from before and after the overall experience were analyzed for this objective. One model was developed for the analysis of variables from the human test, the suddenness test and the novel object test:

$$Y_{ijklm} = \mu + group_i + sequence(group)_{ij} + block(group)_{ik} + cow(group\ block\ sequence)_{ijkl} + time_m + time{*}sequence(group)_{ijm} + e_{ijklm}$$

Where: Y_{ijklm} is the dependent variable, which is the outcome measure of the l^{th} cow from the k^{th} block from the i^{th} group with j^{th} sequence at the m^{th} time. The $group_i$ is the fixed effect of i^{th} group (i= 1, 2). The $sequence(group)_{ij}$ is the fixed effect of j^{th} sequence (j= 1 to 3) within the i^{th} group. The $block(group)_{ik}$ is the fixed effect of the k^{th} block (k= 1 to 5). The cow(group block $sequence)_{ijkl}$ is the random effect of l^{th} cow (l= 1) within the i^{th} group, k^{th} block and j^{th} sequence. The $time_m$ is the fixed effect of the m^{th} time (m= before, after). The $time*sequence(group)_{ijm}$ is the fixed effect of the interaction between the m^{th} time and the j^{th} sequence. Finally, e_{ijklm} is the random error.

To account for the repeated measures, the best fitting covariance structure (among Auto-Regressive (1) or AR1, Compound Symmetry or CS, and Unstructured or UN) was selected for each outcome measure following the BIC fit statistics assessment. Normality of residuals for each outcome measure was tested using PROC MIXED and PROC UNIVARIATE procedures.

3.2. RESULTS

3.2.1. Objective 1 – Determine the motivation of the cow to go to indoor or outdoor exercise area (trip test)

For the trip test, no effect of treatment was found for the time to put on the halter to the cow ($P = 0.1512$, Ddf = 51.6, F-value = 2.12) and the trip speed ($P = 0.2458$, Ddf = 49.5, F-value = 1.38). Only a numerical difference was found for these measures, with the cows going outside having a smaller time to put on the halter (LSmeans ± SE; 65 ± 3.1 s) and being a bit slower during the trip (1.24 ± 0.152 m/s) compared to the cows going inside (68 ± 2.9 s; 1.31 ± 0.143 m/s). A statistically significant effect of treatment was found for the total speed ($P < 0.0001$, Ddf = 54.8, F-value = 30.84), with cows having access to outdoor exercise (0.36 ± 0.026 m/s) being faster than the cows going to the indoor pen (0.23 ± 0.025 m/s).

For all outcome measures of the trip test (time to put on the halter, trip speed and total speed), there is a statistically significant effect of periods ($P < 0.05$), with the time to put on the halter decreasing and speeds increasing between periods 1 and 2 (Table 3.2.1).

No effect of person was found for the trip speed ($P = 0.2611$) and the total speed ($P = 0.8736$). It is statistically significant for the time spent to put on the halter ($P = 0.0001$), with an average time for person A shorter than person B (Table 3.2.1).

All results referring to the trip test are presented in Supplemental Table 4.

Table 3.2.1. Estimated means (LSmeans ± SE) of period and person effects for time to put on halter (s), trip speed (m/s) and total speed (m/s) in the trip test.

Outcome measures	Period					Person				
	Period 1	Period 2	Ddf	F-value	P-value	Person A	Person B	Ddf	F-value	P-value
Time to put on the halter, s	72 ± 3.0	66 ± 2.6	54.5	5.4	0.0073	56 ± 1.8	76 ± 4.7	49.6	17.83	<0.0001
Trip speed, m/s	0.95 ± 0.151	1.23 ± 0.131	52.4	7.63	0.0012	1.15 ± 0.092	1.40 ± 0.228	46.6	1.29	0.2611
Total speed, m/s	0.25 ± 0.026	0.30 ± 0.022	58.8	3.62	0.0328	0.30 ± 0.013	0.29 ± 0.041	54.7	0.03	0.8736

3.2.2. Objective 2 - Determine the reactivity of the cow in relation to its treatment (experience in the indoor or outdoor exercise area during period 1 only)

3.2.2.1. Human test

In the human test, no effect of treatment was found for the latency, average of all stages (mean) and stages, except for stage 2 ($P = 0.0241$, Ddf = 16, F-value = 4.75). The latter corresponds to the moment the human would make a first step closer to the cow, arms placed alongside his body. Score of reaction (scale: -3 to 3) is more negative in the case of the cows in the stall treatment (-0.39 ± 0.106) followed by cows having access to indoor exercise (-0.04 ± 0.106). Animals in the outdoor treatment have the most positive score of reaction (0.05 ± 0.106). After adjustment for multiple comparisons (Bonferroni), a statistically significant difference was found between these results for the outdoor and the stall treatment cows ($P = 0.0311$). A tendency of difference ($P = 0.0964$) was also found between indoor and stall treatment cows.

No time effect was found for the latency and stages in the human test. Statistically significant effect of time was found for the score average of the five stages or mean ($P = 0.0318$, Ddf = 24, F-value = 5.20). Cows had a lowest score before (-0.50 ± 0.100) as compared to after period 1 of treatment application (-0.31 ± 0.100).

3.2.2.2. Suddenness test

No effect of treatment was found for both reaction and freezing time in the suddenness test ($P > 0.05$). No other effect was found for reaction.

A statistically significant effect of the interaction between treatment and time was found for the freezing time ($P = 0.0242$, Ddf = 24, F-value = 4.36). However, no statistically significant difference between the six values was found after correction for multiple comparisons. Only numerical differences are noticeable. As presented in Figure 3.2.1, freezing time is longer after the period 1 of treatment application compared to before for cows in indoor and outdoor treatment. Before the first period, freezing time for cows in stall treatment was longer than cows in indoor and outdoor treatment. Additionally, freezing time for stall cows reduces over time compared to indoor and outdoor cows, where it is increasing.

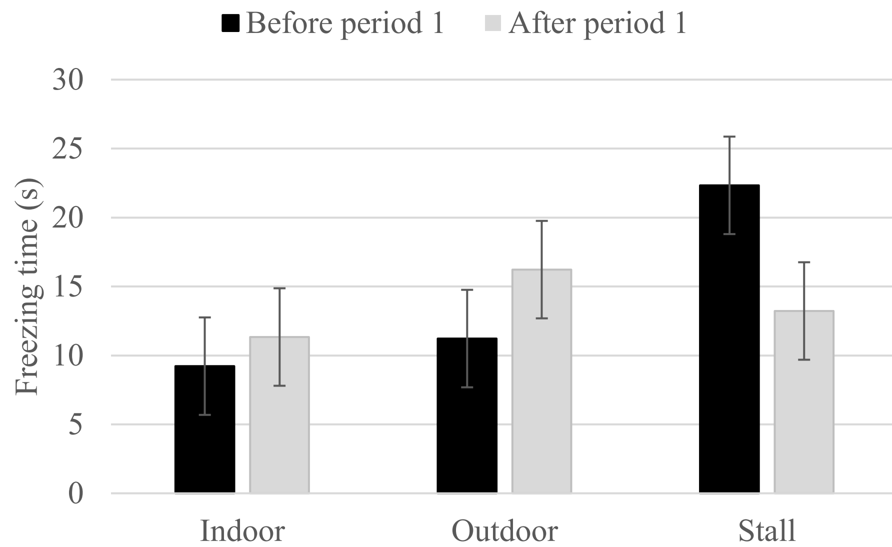

Figure 3.2.1. Freezing time (s) before (■) and after (■) first period of treatment application for indoor, outdoor and stall cows in suddenness test. Bars represent LSmeans and SE. Statistically significant effect of interaction of treatment by time was found for freezing time during suddenness test (P = 0.0242, Ddf = 24, F-value = 4.36). However, no statistically significant difference between the six values was found after correction for multiple comparisons.

3.2.2.3. Novel object test

A statistically significant effect of treatment was found for some of the outcome measures in the novel object test. It was found for the latency to move away from the object (P=0.0478, Ddf = 16, F-value = 3.70). Cows in the stall treatment have the longer latency (18.1 ± 2.29 seconds), followed by cows having access to indoor (17.1 ± 2.29 seconds) and outdoor exercise (10.0 ± 2.29 seconds). Treatment effect was found for the frequency per minute of the distance between one and two heads between the cow and the object (P-value = 0.0315, Ddf = 16, F-value = 4.32) and the time spent at this distance (P-value = 0.0443, Ddf= 16, F-value = 3.81). Outdoor treatment cows have the highest frequency per minute at this distance (3.2 ± 0.32 nb/min), followed by indoor (2.7 ± 0.32 nb/min) and stall treatment (1.9 ± 0.32 nb/min). Time spent at this distance is higher for cows in indoor treatment (20.4 ± 3.45 %), followed by outdoor (15.4 ± 3.45 %) and stall treatment (7.0 ± 3.45 %). Treatment effect was also found for the frequency of eating per minute (P-value = 0.0082, Ddf = 16, F-value = 6.59). Indoor treatment animals have the highest frequency (0.3 ± 0.04 nb/min), followed by outdoor (0.1 ± 0.04 nb/min) and stall treatment cows (0.1 ± 0.04 nb/min).

A statistically significant effect of the interaction between treatment and time was found for the time spent in interaction with the object (P= 0.0293, Ddf = 24, F-value = 4.10). An effect of this interaction was also found for the latency to lick (P = 0.0051, Ddf = 24, F-value = 6.63) and the licking frequency per minute (P = 0.0203, Ddf = 24, F-value = 4.61). In these three outcome measures, tendencies or significant differences were found between outdoor treatment cows before the first period of treatment application and other groups of cows (Figure 3.2.2 a), b) and c)). The effect of the interaction between treatment and time is statistically significant for the time spent eating (P = 0.0058, Ddf = 24, F-value = 6.42). In the case of this outcome measure, a statistically significant difference was found between indoor treatment cows before the first period of treatment application and all other groups of cows (Figure 3.2.2 d)).

As shown in Table 3.2.2, a statistically significant effect of time was found for many latencies, frequencies of behaviors per minute and frequencies of the distances from the object per minute (P < 0.05). There is also a time effect for the time spent looking at the object (P = 0.0018). Latencies on most of the behaviors observed are higher after the first period of treatment application than before. Frequencies of behaviors per minute related to the object are smaller after period 1 compared to before. The same pattern can be observed with the frequencies of the distances from the object. Additionally, animals are spending much less time looking at the novel object after the first period of treatment application. Finally, between before and after period 1, there is a significant increase of the time spent in rumination (P = 0.0325) and resting (P = 0.0340).

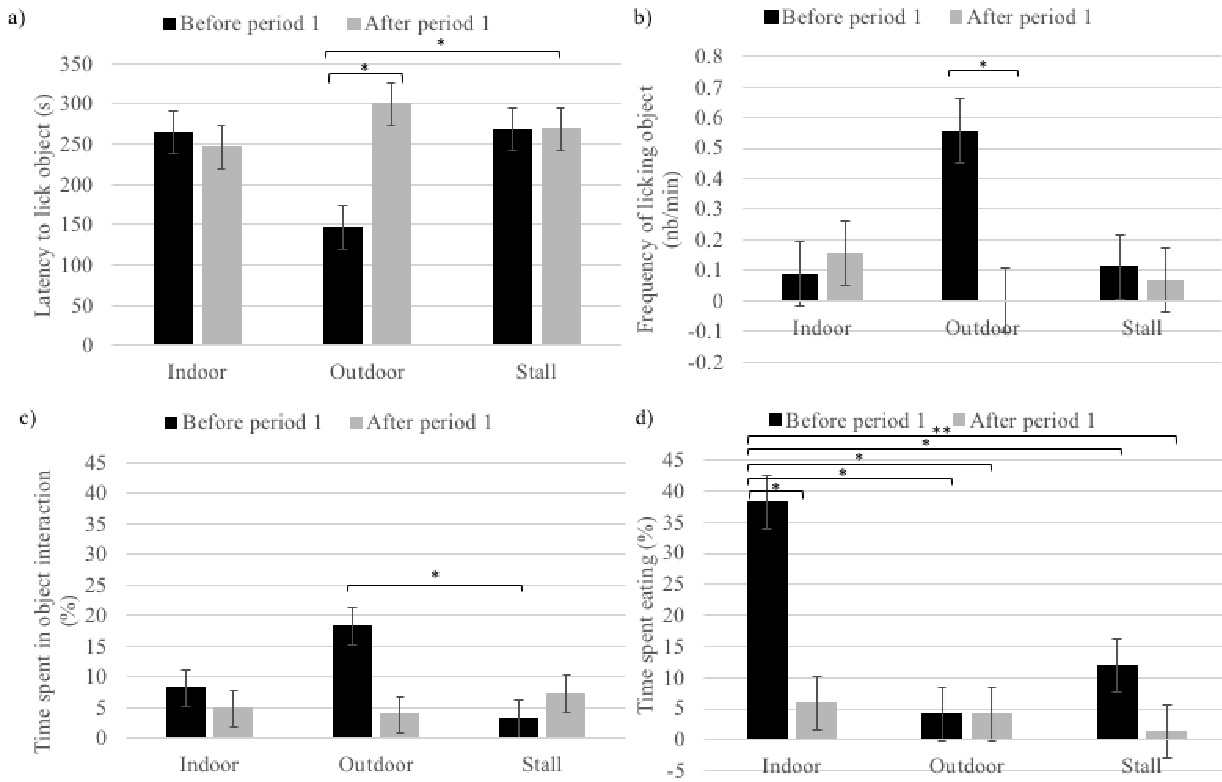

Figure 3.2.2. Latency to lick the object (a), frequency of licking the object (b), time spent in object interaction (c) and time spent eating (d) during the novel object test for each treatment (indoor, outdoor and stall) before (■) and after (■) first period of treatment application. Bars represent LSmeans and SE. Significance of $P < 0.05$ denoted by * and $P < 0.0001$ denoted by **.

Table 3.2.2. Estimated means (LSmeans ± SE) for latencies, frequencies (frq) and times spent performing different behaviors observed in novel object test for time effect before and after period 1 of treatment application and significance of time, treatment and the interaction between treatment and time.

Outcome measures	Time					Treatment			Treatment*time		
	Before period 1	After period 1	Ddf	F-value	P-value	Ddf	F-value	P-value	Ddf	F-value	P-value
Latency approach, s	10.0 ± 1.08	21.2 ± 10.62	26.4	1.15	0.2937	25.2	1.20	0.3168	26.4	1.55	0.2305
Latency move away, s	12.1 ± 1.03	18.0 ± 2.55	24	4.38	0.0472	16	3.70	0.0478	24	1.28	0.2968
Latency sniff, s	50.7 ± 21.90	135.3 ± 21.90	24	7.19	0.0130	16	0.70	0.5113	24	0.19	0.8260
Latency push, s	250.3 ± 16.21	250.4 ± 16.21	24	0.00	0.9936	16	0.93	0.4131	24	1.93	0.1669
Frq look, nb/min	1.3 ± 0.09	1.0 ± 0.09	24	8.59	0.0073	16	0.27	0.7665	24	0.20	0.8203
Frq approach, nb/min	1.8 ± 0.13	1.1 ± 0.13	24	25.33	<0.0001	16	2.84	0.0882	24	0.91	0.4155
Frq move away, nb/min	1.8 ± 0.13	1.1 ± 0.13	24	19.75	0.0002	16	2.89	0.0846	24	0.75	0.4817
Frq sniff, nb/min	0.6 ± 0.09	0.4 ± 0.09	24	3.01	0.0958	16	0.08	0.9213	24	0.47	0.6302
Frq push, nb/min	0.2 ± 0.06	0.1 ± 0.06	24	0.48	0.4929	16	0.33	0.7247	24	3.03	0.0671
Time look, %	40.1 ± 3.61	22.5 ± 3.61	24	12.33	0.0018	16	1.73	0.2092	24	1.68	0.2074
Frq touch, nb/min	1.1 ± 0.17	0.8 ± 0.17	24	2.42	0.1329	16	1.83	0.1920	24	1.57	0.2296
Frq < 1 head, nb/min	2.6 ± 0.31	1.8 ± 0.31	24	3.89	0.0601	16	2.84	0.0877	24	1.10	0.3479
Frq 1 to 2 heads, nb/min	3.2 ± 0.26	2.1 ± 0.26	24	8.89	0.0065	16	4.32	0.0315	24	0.52	0.5989
Frq > 2 heads, nb/min	2.3 ± 0.21	1.6 ± 0.21	24	10.67	0.0033	16	0.27	0.7650	24	0.13	0.8762
Frq maximum, nb/min	0.6 ± 0.15	0.5 ± 0.15	24	0.38	0.5448	16	0.80	0.4682	24	0.08	0.9209
Time ruminating, %	11.7 ± 5.73	29.7 ± 5.73	24	5.15	0.0325	16	1.13	0.3470	24	0.70	0.5083
Time resting, %	2.5 ± 1.70	16.8 ± 5.81	24	5.06	0.0340	21.3	0.15	0.8598	24	0.28	0.7575

3.2.3. Objective 3 – Determine the reactivity of the cow in relation to the overall experience (periods 1 and 2)

3.2.3.1. Human test

A statistically significant effect of time ($P < 0.05$) was found for stages 2 to 5 and the average of all stages (mean). As shown in Figure 3.2.3, scores for these measures were higher after the overall experience. Stage 1 values are not presented since its score is supposed to be zero, which was confirmed with no significant difference over any effect.

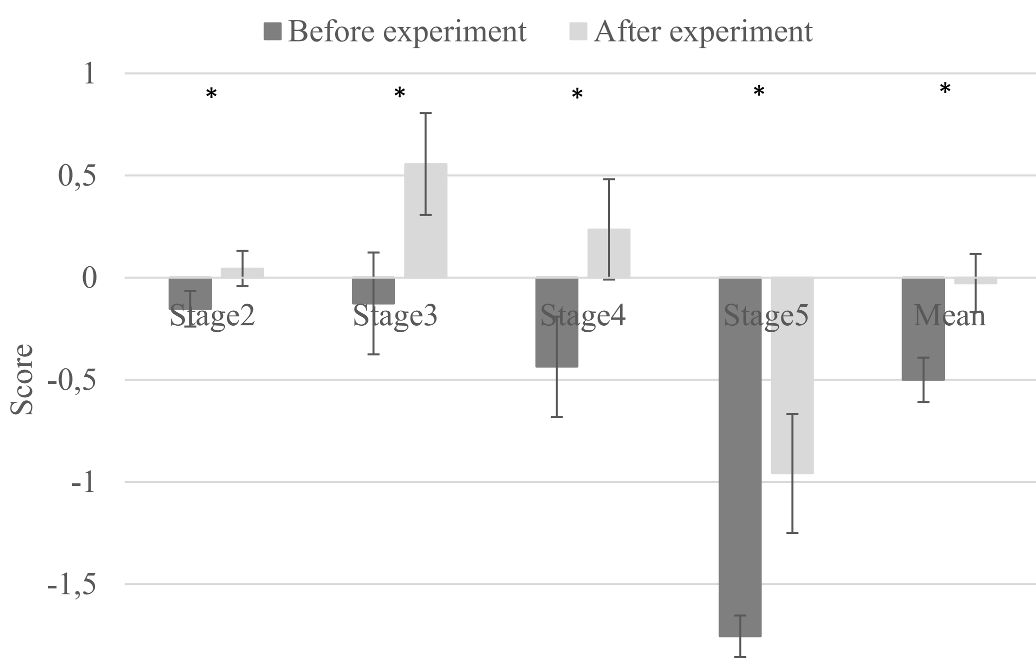

Figure 3.2.3. Score at each stage and average of all stages (mean), obtained in the human test for cows before ( ) and after ( ) overall experience. Bars represent LSmeans and SE. Significance of $P < 0.05$ denoted by *. Significant time effect was found for all measures presented in this figure.

3.2.3.2. Suddenness test

No effect of time was found for reaction and freezing time during suddenness test ($P > 0.05$).

3.2.3.3. Novel object test

As shown in Table 3.2.3, a statistically significant effect of time ($P < 0.05$) was found for the latency to move away from the object, frequencies of looking, approaching and moving away from the object. A time effect was also found for frequencies of the distances more than two heads and stretched maximum between the cow and the object ($P < 0.05$). A statistically significant effect of time ($P < 0.05$) was found for the time spent resting. Latency to move away from the object is longer after the overall experience. Time spent resting also increases. On the other hand, frequencies of looking, approaching and moving away from the object are decreasing after the overall experience. The same pattern is observed for the frequencies of the two distances from the object (more than two heads and stretched maximum).

No time effect was notably found for the time spent looking at the novel object and the time spent in object interaction ($P > 0.05$; Table 3.2.3).

Table 3.2.3. Estimated means (LSmeans ± SE) for latencies, frequencies (frq) and times spent performing different behaviors observed in novel object test for time effect for before and after overall experience and significance of time and the interaction between sequence and time.

Outcome measure	Time					Sequence*Time		
	Before experiment	After experiment	Ddf	F-value	P-value	Ddf	F-value	P-value
Latency approach, s	9.8 ± 1.78	12.5 ± 1.78	21	1.24	0.2784	21	1.64	0.1925
Latency move away, s	11.8 ± 1.84	19.2 ± 1.84	21	8.86	0.0072	21	1.62	0.1979
Latency sniff, s	47.6 ± 19.00	90.8 ± 19.00	21	2.78	0.1105	21	1.62	0.1982
Latency lick, s	226.7 ± 18.04	250.5 ± 18.04	21	1.29	0.2687	21	1.07	0.4029
Latency push, s	253.9 ± 20.09	228.0 ± 20.09	21	1.87	0.1862	21	1.01	0.4352
Frq look, nb/min	1.3 ± 0.07	0.8 ± 0.07	21	17.56	0.0004	21	0.73	0.6113
Frq approach, nb/min	1.8 ± 0.14	1.2 ± 0.14	21	8.27	0.0091	21	1.46	0.2434
Frq move away, nb/min	1.8 ± 0.13	1.1 ± 0.13	21	9.71	0.0052	21	1.39	0.2691
Frq sniff, nb/min	0.6 ± 0.10	0.5 ± 0.10	21	0.63	0.4360	21	1.62	0.1973
Frq lick, nb/min	0.2 ± 0.08	0.2 ± 0.08	21	0.38	0.5457	21	1.47	0.2409
Frq push, nb/min	0.2 ± 0.11	0.3 ± 0.11	21	1.16	0.2937	21	0.41	0.8364
Time look, %	39.9 ± 4.86	31.5 ± 4.86	21	1.40	0.2508	21	0.72	0.6171
Time interaction, %	9.5 ± 3.18	12.5 ± 3.18	21	0.52	0.4770	21	0.98	0.4552
Frq touch, nb/min	1.1 ± 0.19	1.2 ± 0.19	21	0.11	0.7489	21	2.83	0.0417
Frq < 1 head, nb/min	2.6 ± 0.34	2.4 ± 0.34	21	0.35	0.5627	21	3.59	0.0168
Frq 1 to 2 heads, nb/min	3.2 ± 0.28	2.4 ± 0.28	21	4.24	0.0521	21	2.79	0.0437
Frq > 2 heads, nb/min	2.3 ± 0.18	1.4 ± 0.18	21	9.99	0.0047	21	0.84	0.5344
Frq maximum, nb/min	0.6 ± 0.14	0.2 ± 0.06	21	9.32	0.0060	21	1.35	0.2812
Time eating, %	19.0 ± 4.30	14.9 ± 4.30	21	0.62	0.4390	21	1.07	0.4056
Time ruminating, %	11.6 ± 5.91	20.2 ± 5.91	21	0.75	0.3963	21	0.71	0.6252
Time resting, %	2.4 ± 1.87	23.0 ± 7.14	21.1	7.48	0.0124	21.1	0.32	0.8961

3.3. DISCUSSION

3.3.1. Objective 1 – Determine the motivation of the cow to go to indoor or outdoor exercise area (trip test)

The time to put on the halter decreased and speeds (trip and total) increased over periods of treatment application. This may suggest that six outings per period of three weeks were enough for cows to be habituated and learn how to go to exercise areas. Cows' capacity to quickly learn a new procedure was also shown for example by Jacobs and Siegford (2012), when transitioning from being milked by a stockperson from a milking parlor towards being so from an automatic milking system (AMS). In the AMS, the cows need to learn to access the milking robot by themselves to be milked. In their case, over 60% of the herd was milking voluntarily within a week, over 75 % after two weeks and 95% after one month.

A treatment effect (i.e., being led to the indoor or outdoor exercise area) was found for the total speed. This outcome measure includes the time to put on the halter and the duration of the trip and is divided by the distance from cows' home stall to the exercise area. We hypothesized that the habituation shown by the period effect (i.e., time to put on the halter decreased and speeds increased over the periods of treatment application) might be accompanied by motivation of the cows. Outdoor treatment cows have a faster total speed than indoor treatment cows, probably because of a higher motivation to go outside than inside. This could be explained by a more enriched environment (example: textural due to presence of snow on the ground or visual due to multiple stimuli such as trees) and different from what the cow usually sees inside the barn. Another explanation could be that faster total speed for outdoor cows could be due to a longer distance to access the outdoor exercise area. However, as mentioned in the review of Smid et al. (2020), the motivation of cows to access alternative (to pasture) outdoor areas should undergo further investigation.

This difference between indoor and outdoor treatment cows is not shown by the two other measures we collected, the time spent to put on the halter and the trip speed. That might be because the time to put on the halter was highly influenced by the person manipulating the cow. Here, person A had the shortest time. This has to be interpreted carefully. In fact, person A was the handler for the trip test 90 % of the time compared to 10 % of the time for person B. Thus, person B did not obtain as much experience with the procedure and may have been less at ease to put on the halter to cows. For example, Boivin et al. (2003) mentioned that dairy cows were more or less easy to handle depending on the characteristics of the stockperson, such as level of confidence, of experience and degree of agitation.

Only a numerical difference was found between indoor and outdoor treatment cows for the trip speed with cows going outside being a bit slower during the trip than the cows going inside. This is not according to our hypothesis i.e., cows going outside should be more motivated and therefore would walk more quickly than cows going inside, as shown by the total speed being faster for outdoor cows. The complexity of the physical environment of the walkway used for leading cows outdoors might explain this lack of significant difference in trip speed or even outdoor cows being slower than indoor cows and the discrepancies with our total speed results. The environment to go to the outdoor exercise areas had more sharp curves compared to the straight line for the indoor exercise area. It was documented by Grandin's research that cattle can refuse to walk over a shadow and go through a change in flooring surface. Similarly, blinding light is unattractive for cattle (Grandin, 1999). In our experimental conditions, three types of flooring surfaces (concrete, rubber and snow) were present in the environment to lead cows outdoors, compared with only one type (and no change) in the case of the environment leading to the indoor exercise area. A small cabin was also constructed between the barn and the outside to protect the entrance area from snow falls. This might have created shadow followed by a blinding light which was produced by the sun reflecting on snow. These examples show that there can easily be a challenge associated with the environment used for leading cows outdoors and that the latter should carefully be considered. In our case, the environment was originally not designed to lead cows outdoors and shows the importance of taking into consideration these factors when repurposing a barn to provide outdoor access, as it could negatively impair the experience of both the cows and the handler.

3.3.2. Objective 2 – Determine the reactivity of the cow in relation to its treatment (experience in the indoor or outdoor exercise area during period 1 only)

For the human test, we did not find any treatment*time interaction for the latency to touch the human, all stages and the average of stages (mean) neither a treatment effect except for the score from stage 2, where we found a treatment effect. It is not according to our hypothesis.

We hypothesized an improvement in cows' reactivity to humans after the application of the treatment (i.e., decrease of latency and increase of scores indicated by treatment*time interaction). The main effect of treatment may have diluted the interaction treatment*time: the difference between treatment groups was already present before the first period of treatment application which may have contributed to the lack of difference between groups after treatment application. The absence of difference could also be due to the frequency or duration of treatment application (outings). Six outings in three weeks (indoor and outdoor) might not be enough to notice a significant effect in cow-human relationship for our trial cows. In Aigueperse and Vasseur (2021) experiment, in which the cows were lead outdoors with more outings per week (five instead of two) for a longer period of time (twelve weeks instead of three), a positive effect on cow's relationship with humans on their winter trial was found. In the case of our experiment, more outings, including necessarily more manipulations, might have influenced cow-human relations.

In the suddenness test, we found a significant treatment*time effect with a numerical increase of the freezing time observed for both indoor and outdoor cows (this difference is not significant after correction for multiple comparisons). No interaction or treatment, nor time effect on the reaction score, was found. We hypothesized that the animals that have access to outdoor or indoor exercise areas could adapt better to sudden events, considering the provision of various stimuli provided, especially in the outdoor exercise area (Sackett et al., 1999). The increase of freezing time is difficult to analyze alone (no effect was found on the reaction score). A positive interpretation of the increase in freezing time could be that the animals might be more curious after compared to before the first period of treatment application. However, a negative interpretation could be that cows might not have had enough time to learn totally about inside and outside enrichment. The cows were probably still exploring and learning their new environment and may not have learned how to react positively to sudden events, which may explain why their reaction (measured through the reaction score) did not change over time, but more time was spent looking at the object (measured through freezing time).

For the novel object test, we found few variables with significant treatment*time interaction. When looking at the two-by-two comparisons, we found that the differences between treatments were before (and not after) the treatment application period. The second most important result for the novel object test is the time effect for many variables, showing that cows became habituated to the test across even when the test was only applied twice over a 1-month period. Cows from all treatments after the first period of treatment application are interacting less early and less often with the novel object. Also, the animals are not moving as much between different distances and spending less time looking at the object. After the first period, all treatment cows do not seem to show as much interest in the novel object. Our main hypothesis for the novel object test was to observe improved reactions (i.e., decrease of fear reaction such as decreased distances between object and cows, and possibly improved curiosity such as increased frequencies of interactions e.g., licking). Our results show that first, a difference between treatments exists before the application of treatments and second, the object was not novel the second time the test was performed for each animal (i.e., at the end of the first period of treatment application). Therefore, we were not able to validate or invalidate our hypothesis. Regarding the habituation of all cows to the test, three reasons might explain this. First, the interval between testing was probably too short (around one month) and the test was too long (five minutes with the object). Second, the ball having different colors and patterns was probably not sufficient. Third, depending on the placement of the cow, it could be that some were able to see the object of the neighbor on the same day or some days before their own test. Even though objects of the neighbors were of different colors (and sometimes patterns), it was probably not sufficient to maintain novelty. Kappel et al. (2017) observed a possible habituation by presenting the same object on three consecutive days. To overcome this issue, Hedlund and Løvlie (2015) used a new object for each observation.

3.3.3. Objective 3 – Determine the reactivity of the cow in relation to the overall experience (periods 1 and 2)

While the individual effect of the first and second period of application on the overall cow reaction could not be separated, our aim in reapplying our three tests after the second period of treatment application was to evaluate the effect of six weeks of trial enrollment (i.e., time effect), exposure to different procedures and handling by humans on cow's reactivity. For the human test, we found a significant time effect for most of our variables: all enrolled cows have an increase of scores over time (i.e., cows approach humans instead of avoiding humans during the test). This is according to our hypothesis to observe an improvement in cows' reactivity to humans after the overall experience. The first explanation is that the animals became habituated to the procedures of outing (and therefore react less to humans). The second explanation is that cows may perceive the outing positively, which corroborates results from objective 1 that cows are motivated to get out of the stalls both to get indoors or outdoors. Either way, the overall experience was positive in terms of human-animal relationships. This result is favorable because it can facilitate handling and possibly decrease risks of injuries during handling. Indeed, fear-related reactions from animals can be dangerous for both human and cow and associated with time waste or economic loss for the farmer (Boissy and Bouissou, 1995; Lewis and Hurnik, 1998).

For the suddenness test, we did not find a time effect. Reactivity of the cows related to suddenness did not change after the overall experience. It is not according to our hypothesis that the animals could adapt better to sudden events after being exposed to various stimuli. We could reiterate some of the explanations of results observed after a first treatment application (objective 2). It is possible that cows did not get enough exposure to new environments i.e., not enough outings to have an influence on this factor, or sudden events may not have (or few) occurred during the few outings. Additionally, the inside exercise area is close to the stalls, so the sudden events that occurred in the exercise areas may not have been different from what the cows experienced in their home stall.

For the novel object test, we found a time effect on a number of variables. When the test was reapplied after the overall experience, animals seemed to be less uncertain when faced with the ball, because they were doing less back and forth and had longer periods of observations. In fact, the first time the cows moved away after looking at the object (measured by the latency to move away), was later after the experiment compared to before. Also, the animals were spending an equal time interacting with the object and looking at it. As mentioned earlier, our main hypothesis for the novel object test was to observe improved reactions. There is a possibility that the cows habituated to the test as it was shown after the first period of treatment application (objective 2), so it cannot be concluded if cows are less reactive to novelty. However, compared to objective 2 where cows were not interested in the object after one period of treatment application, the animals after the overall experience (two periods of treatment application) were still interested and might show curiosity. The curiosity might come from the longer exposure cows had in the exercise areas. In those enriched environments, cows learned to see more things that they were not used to. The outings might have been sufficiently enriching to arouse cows' curiosity of novel objects. As mentioned by Berlyne (1955) in his research conducted with rats on the arousal of curiosity, an environment presenting numerous and complex stimuli is explored more than one with only few and simple stimuli.

In conclusion, the trip test allowed us to demonstrate that after approximately twelve outings in two periods of three weeks, dairy cows were already habituated to go to exercise areas. We showed that cows were also more motivated to go outdoors than to the indoor exercise area. Based on our results, it is clear that the handler has an effect on the ease of handling. Both after one period of treatment (objective 2) and the overall experience (objective 3), the animals might have habituated to the novel object test. Additionally, after the overall experience (i.e., six weeks of trial enrollment, exposure to different environments and procedures, and handling by humans), we have been able to observe a positive effect on human-animal relationship (human test) and possibly arousing curiosity (novel object test). Allowing exercise access to cows might in fact facilitate handling and decrease fear reactions, which benefit both human and animal welfare.

3.4. CONCLUSION

The general objective of this thesis was to review the behavioral tests currently used in existing literature to examine bovines' emotions and to determine the influence of indoor and outdoor exercise access on the motivation and reactivity of cows housed in tie-stalls. In the literature review, we have seen that different types of tests are commonly used to study emotions: arena, human, novel object and frustration/excitement tests. These tests differ from each other in a few of their conditions, including the physical environment where the test is performed, conditions before and during the test, and variables measured. Identification of cow's emotions is mostly effected using behavioral measurements. One of the challenges associated to behavioral tests, as for the arena and novel object tests, is to keep novelty when repeating them over time. In fact, this phenomenon of habituation to the test stimulus has been one issue which we experienced in our use of the novel object test during our study. Further research should investigate into which types of objects could be used interchangeably when repeating these types of tests over time while avoiding losing the novelty aspect critical to the success of the test. Also, contrary to our initial hypothesis, our results showed no improvement in cow reactivity related to suddenness (after one period of treatment and the overall experience). Allowing a higher frequency and/or a longer period of treatment application (outings) associated with sudden events different from what the cows are used to, might have improved cows' reactivity. Further research should be conducted with more outings per week (with more sudden events, by using a more enriching environment) and/or a longer period of time, to observe the effect on cows' reactivity to suddenness.

Most of the behavioral tests presented in the literature review have similarities, as they aim to look at cows' reactivity (i.e., the cows' capacity to perceive and react to potentially anxiogenic situations). Most of the tests reported in the literature investigate negative emotions (e.g., fear of humans). Yet, even in tests primarily examining negative emotions, it seems possible to look at positive outcomes, such as curiosity, by going further in the interpretation of the results obtained after analysis. This is what was done in the case of the novel object test in our experimental study. We found that cows were possibly more curious (as measured by the novel object test) at the end of the overall experience after exposure to different environments, procedures and handling by humans over six weeks of trial. It is also possible to look at positive outcomes directly: such is what motivation tests are designed for. Not only did we find that cows were habituated to be led indoors or outdoors at the end of the experience, but our trip test also showed that cows were more motivated to go outdoors than to the indoor exercise area. The improvement of cows' reactivity to humans, as shown by the human test results (i.e., cows approached instead of avoiding humans during the test, then might perceive the outings positively) after the overall experience, might be further supporting a higher motivation to the animals to go to indoors or outdoors.

Our results shed new light on the potential the provision of exercise access to dairy cows has to improve cow welfare, by showing that offering indoor or outdoor access may arouse curiosity and enhance motivation in cows. The positive relationship between cows and humans resulting from this experiment is beneficial for both (specifically decrease cows' fear reactions and facilitate handling).

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SUPPLEMENTAL MATERIAL

Supplemental Text 1. Standard operating procedure for maintenance of exercise areas, decision tree for outings

Outside

A decision tree was created in order to decide if cows were going in the outdoor exercise area. Firstly, the experimenter measured outside air temperature by using a wind speed/temperature instrument in the exercise area. Based on the temperature and the wind velocity collected, the windchill index was established using the windchill index chart (Supplemental Table 1). If the windchill index was ≤ -25 °C, the cows would not get their treatment for the day, but if the windchill index was > -26 °C, cows might be able to go outside depending on the next steps. Secondly, the experimenter would verify if it is raining and if it is freezing rain. If "no" was answered to those two questions, then the next question was: is it snowing and is it possible to see the exercise yard from the exit door? If it was not snowing or if the exercise yard could be seen, then the next question was if the walking surface was slippery. The surface was considered slippery if one cannot walk at a normal pace without a feeling of slipperiness underfoot and/or without having to brace himself to the fence in the alleys and exercise yard. If too slippery, experimenter had to proceed with a clean-up. The alley was then scraped down to concrete in order to break and remove slippery material, and sand was added as an abrasive after cleaning up. In the exercise area, slippery material was also removed, but cedar mulch was added instead of sand. If the walking surface was still slippery, cows couldn't go outside on that day. But, if the surface was not slippery anymore, the next question regarded the amount of sufficient snow to reach the teats of the cow with the lowest udder. If it was the case, snow was removed in alleys and exercise areas. When meeting all criteria, a cow could go outside.

Inside

After each day of outing in the inside exercise area, wet and dirty bedding were removed from the area.

Supplemental Table 1. Windchill index chart used in decision tree for outings

		Air temperature (°C)							
		-30	-25	-20	-15	-10	-5	0	5
Air velocity (km/h)	0	**-30**	**-25**	-20	-15	-10	-5	0	5
	5	**-36**	**-30**	-24	-19	-13	-7	-2	4
	10	**-39**	**-33**	**-27**	-21	-15	-9	-3	3
	15	**-41**	**-35**	**-29**	-23	-17	-11	-4	2
	20	**-43**	**-37**	**-30**	-24	-18	-12	-5	1
	25	**-44**	**-38**	**-32**	**-25**	-19	-12	-6	1
	30	**-46**	**-39**	**-33**	**-26**	-20	-13	-6	0
	35	**-47**	**-40**	**-33**	**-27**	-20	-14	-7	0
	40	**-48**	**-41**	**-34**	**-27**	-21	-14	-7	-1
	45	**-48**	**-42**	**-35**	**-28**	-21	-15	-8	-1
	50	**-49**	**-42**	**-35**	**-29**	-22	-15	-8	-1

Supplemental Text 2. Standard operating procedure to prepare for outing

This procedure was followed by experimenter and handlers to prepare outing of cows.

Steps

1- Scrub back of the stall to clean manure and urine. Scrub gutter to facilitate fitting of panels.

2- Untie tails from strings.

3- Sweep alley and spread non-slip.

4- Install chains around the alley and install panels in the gutter behind the appropriate cows (cows going to exercise areas).

5- Have handler put on the halter on the cow.

Supplemental Text 3. Standard operating procedure for leading cow to the exercise area

This procedure is followed by handlers every time a cow is led to an exercise area, even during observations for the trip test.

Handler puts on a halter on the cow and ties the loose end of the rope of the halter on the collar. Then, the person will unclip the chain of the cow and lead her by the halter to the exercise area. During the whole procedure, an experimenter is following the animal by keeping a distance of at least five meters behind the cow.

Steps to encourage cow to walk forward when she stops walking:

1- Handler stops and waits five seconds for the cow to walk by herself again. If the cow does not react, he moves on to the next step.

2- Handler pulls the cow with the halter using one hand. He waits five seconds. He repeats the step three times. If the cow does not react, he moves on to the next step.

3- Handler pulls the cow with the halter using two hands. He waits five seconds. He repeats the step three times. If the cow does not react, the experimenter moves on to the next step.

4- Experimenter makes noises and waves arms. He waits five seconds. If the cow does not react, he moves on to the next step.

5- Experimenter gently pats the top of the udder. He waits five seconds. If the cow does not react, he moves on to the next step.

6- Experimenter gently taps the side of the leg. He waits five seconds. If the cow does not react, he moves on to the next step.

7- Experimenter gently pushes the bottom of the cow using his shoulder. He waits five seconds. If the cow does not react, he moves on to the next step.

8- Experimenter gently taps dewclaw with one foot. He waits five seconds. If the cow does not react, he moves on to the next step.

9- Experimenter prods spinal column with a pencil. He waits five seconds. If the cow does not react, he repeats this step until the cow starts walking again.

Steps to stop the cow when it is too fast:

1- Handler approaches his hands to the head of the cow with the halter for better control.

2- Handler makes noise.

3- Handler taps on the nose.

Supplemental Table 2. Intra-observer repeatability scores for measured variables during continuous video observation of novel object test

Measured variables	Intra-observer (overall average %)[1]
Behaviors	**92**
Before test	89
R1 vs R2	88
R3 vs R2	89
R4 vs R2	89
R5 vs R2	89
R6 vs R2	89
R7 vs R2	89
R8 vs R2	89
Related to novel object	87
R1 vs R2	81
R3 vs R2	71
R4 vs R2	88
R5 vs R2	92
R6 vs R2	92
R7 vs R2	92
R8 vs R2	96
Not related to novel object	100
R1 vs R2	100
R3 vs R2	100
R4 vs R2	100
R5 vs R2	100
R6 vs R2	100
R7 vs R2	100
R8 vs R2	100
Distances	**79**
R1 vs R2	75
R3 vs R2	85
R4 vs R2	80
R5 vs R2	85
R6 vs R2	75
R7 vs R2	80
R8 vs R2	75

[1] Repeatability (R) was performed with frequencies of behaviors or distances. Based on the difference in the number of behaviors or distances observed in two exercises, a percentage of agreement was developed and classified within 3 categories: red (0 to <60% of agreement), yellow (≥ %60 to < 80% of agreement) and green (≥ %80 of agreement). The percentage of agreement in the green category was averaged for all behaviors and distances to get the overall average. Seven repeatability exercises were performed for each measure. Exercises were done by one observer with four different cows (four recordings). Dates of exercises are: December 1st, 2020 (R1 vs R2); January 7th, 2021 (R3 vs R2); January 10th, 2021 (R4 vs R2); January 27th, 2021 (R5 vs R2); February 13th, 2021 (R6 vs R2); March 9th, 2021 (R7 vs R2); March 31st, 2021 (R8 vs R2).

Supplemental Table 3. One day of schedule for behavioral tests and other measures

Hours	Experimenter tasks	Barn staff tasks and restrictions
8h45-9h10	Prepare outing for companion + AM1 J5-J6 cows + anticipation/frustration cows (AM1 J3-J4)	-- No restriction
9h10-9h20	Install cameras AM1 J3-J4 cows for anticipation/frustration	Put on halter AM1 J3-J4 cows for anticipation/frustration
9h20-9h30	Help lead cows to areas	Lead companion + 1 cow to outdoor area + 1 cow to indoor area (AM1 J5-J6 cows)
9h30-9h45	9h35-9h45: Recording of anticipation/frustration AM1 J3-J4 cows	Note: do not disturb cows before tests
Session 1 9h45-10h20	Behavioral tests - Human test: 10 min - Novel object test: 30 min	Note: do not disturb cows
10h20-10h35	Help lead back cows Observation cleanliness at trip back	Lead back AM1 J5-J6 cows except for companion Halter removal
10h35-10h40	Prepare outing for AM2 J5-J6 cows + anticipation/frustration cows (AM2 J3-J4)	-- No restriction
10h40-10h50	Install cameras AM2 J3-J4 cows for anticipation/frustration	Put on halter AM2 J3-J4 cows for anticipation/frustration
10h50-11h00	Help lead cows to areas	Lead 1 cow to outdoor area + 1 cow to indoor area (AM2 J5-J6 cows)
11h05-11h15	Recording of anticipation/frustration AM2 J3-J4 cows	Note: do not disturb cows
11h15-11h30	--	Halter removal
11h30-11h40	Behavioral test - Sudden test	Note: do not disturb cows
11h50-12h05	Help lead back cows Observation cleanliness at trip back	Lead back AM2 J5-J6 cows + companion
12h05-12h15	Remove chains and panels	Note: do not disturb cows before tests
Session 2 12h15-12h55	Behavioral tests - Human test: 10 min	Note: do not disturb cows

	- Novel object test: 30 min	
13h15-13h40	Prepare outing for companion + PM J5-J6 cows + anticipation/frustration cows (PM J3-J4)	-- No restriction
13h40-13h50	Install cameras PM J3-J4 cows for anticipation/frustration	Put on halter PM J3-J4 cows for anticipation/frustration
13h50-14h00	Help lead cows to areas	Lead companion + 1 cow to outdoor area + 1 cow to indoor area (PM J5-J6 cows)
14h05-14h15	Recording of anticipation/frustration PM J3-J4 cows	Note: do not disturb cows
14h15-14h25	Behavioral test - Sudden test	Note: do not disturb cows
14h30-14h55	--	Halter removal
14h50-15h05	Help lead back cows Observation cleanliness at trip back	Lead back PM J5-J6 cows + companion
15h05-15h15	--	Note: do not disturb cows before tests
Session 3 15h15-16h15	Behavioral tests - Human test: 10 min - Novel object test: 30 min - Sudden test: 10 min	Note: do not disturb cows
16h10-16h30	Remove chains, panels, non-slip, etc. Install objects for Monday	-- No restriction
16h45-17h45	SLS J5-J6 cows	Note: do not manipulate animal or pass in front of camera.

Supplemental Table 4. Effects of block, treatment, period, week(Period), person and interaction between treatment and week for trip test variables time to put on the halter (s), trip speed (m/s) and total speed (m/s).

Outcome measure	Block			Treatment			Period			Week(Period)			Person			Treatment*Week		
	Ddf	F-value	P-value	Ddf	F-value	P-value	Ddf	F-value	P-value	Ddf	F-value	P-value	Ddf	F-value	P-value	Ddf	F-value	P-value
Time to put on the halter, s	17	3.43	0.0156	51.6	2.12	0.1512	54.5	5.4	0.0073	45.1	4.59	0.0153	49.6	17.83	0.0001	48.3	2.05	0.1017
Trip speed, m/s	14.3	0.52	0.8240	49.5	1.38	0.2458	52.4	7.63	0.0012	42	0.33	0.7235	46.6	1.29	0.2611	47.2	1.11	0.3618
Total speed, m/s	16.8	1.39	0.2716	54.8	30.84	< 0.0001	58.8	3.62	0.0328	48.5	0.33	0.7188	54.7	0.03	0.8736	46.6	1.61	0.1867

APPENDIX

Appendix Table 1. Estimated means (LSmeans ± SE) for frequencies (frq) and times spent performing different behaviors observed before the novel object test (model 1/objective 2) for time effect before and after period 1 and significance of time, block, treatment and the interaction between treatment and time.[1]

Outcome measure	Block			Treatment			Time					Treatment*Time		
	Ddf	F-value	P-value	Ddf	F-value	P-value	Before	After	Ddf	F-value	P-value	Ddf	F-value	P-value
Frq eating, nb/min	16	0.91	0.5301	16	0.10	0.9095	0.3 ± 0.06	0.2 ± 0.06	24	2.50	0.1266	24	1.24	0.3074
Frq drinking, nb/min	16	0.67	0.7133	16	0.00	1.0000	0.0 ± 0.01	0.0 ± 0.01	24	0.33	0.5691	24	1.33	0.2824
Frq ruminating, nb/min	16	2.59	0.0500	16	2.91	0.0836	0.0 ± 0.03	0.1 ± 0.03	24	2.64	0.1173	24	1.15	0.3321
Frq resting, nb/min	16	1.82	0.1463	16	0.57	0.5758	0.1 ± 0.03	0.1 ± 0.03	24	0.97	0.3343	24	0.62	0.5456
Frq defecating, nb/min	16	1.09	0.4204	16	0.23	0.7982	0.0 ± 0.01	0.0 ± 0.01	24	0.18	0.6736	24	0.18	0.8349
Frq urinating, nb/min	16	1.00	0.4726	16	1.00	0.3897	0.0 ± 0.01	0.0 ± 0.01	24	1.00	0.3273	24	1.00	0.3827
Frq grooming, nb/min	16	0.57	0.7901	16	0.16	0.8546	0.1 ± 0.04	0.1 ± 0.04	24	3.60	0.0697	24	1.77	0.1926
Frq fighting, nb/min	16	2.35	0.0696	16	2.43	0.1194	0.1 ± 0.02	0.0 ± 0.02	24	2.18	0.1527	24	1.64	0.2157
Frq receiving fighting, nb/min	16	0.62	0.7455	16	1.90	0.1818	0.0 ± 0.02	0.0 ± 0.02	24	0.09	0.7630	24	1.77	0.1923

Frq social grooming, nb/min	16	0.73	0.6650	17.5	1.00	0.3880	0.0 ± 0.01	0.0 ± 0.02	24	1.33	0.2596	24	1.33	0.2824
Frq sniffing other cow, nb/min	16	0.75	0.6491	16	1.00	0.3897	0.0 ± 0.02	0.0 ± 0.02	24	0.00	1.0000	24	1.50	0.2433
Frq sniffing environment, nb/min	16	0.87	0.5637	16	0.31	0.7394	0.2 ± 0.05	0.2 ± 0.05	24	0.07	0.7955	24	0.48	0.6242
Frq standing, nb/min	16	1.33	0.2997	16	0.67	0.5232	0.2 ± 0.02	0.2 ± 0.02	24	0.95	0.3401	24	0.32	0.7322
Frq lying, nb/min	16	1.21	0.3543	16	0.55	0.5850	0.1 ± 0.02	0.1 ± 0.02	24	1.68	0.2067	24	1.37	0.2737
Time standing, %	16	1.47	0.2424	16	0.93	0.4161	78.1 ± 8.18	68.5 ±8.18	24	0.79	0.3817	24	0.88	0.4261
Time lying, %	16	1.47	0.2424	16	0.93	0.4161	21.9 ± 8.18	31.5 ± 8.18	24	0.79	0.3817	24	0.88	0.4261
Time ruminating, %	16	1.70	0.1741	16	1.47	0.2597	11.4 ± 7.34	36.6 ± 7.34	24	6.31	0.0192	24	1.08	0.3549
Time receiving fighting, %	16	0.89	0.5466	23.6	1.02	0.3748	0.1 ± 0.06	1.0 ± 1.00	24	0.98	0.3333	24	1.17	0.3276
Time eating, %	16	1.14	0.3887	16	1.61	0.2297	51.9 ± 7.54	29.5 ± 7.54	24	4.33	0.0483	24	0.48	0.6264
Time drinking, %	16	0.75	0.6513	17.3	0.17	0.8411	0.8 ± 0.60	0.2 ± 0.25	24	0.69	0.4133	24	1.15	0.3324
Time resting, %	16.3	1.14	0.3888	16.4	0.53	0.5998	7.1 ± 3.18	29.5 ± 8.90	25.7	5.65	0.0252	25.7	0.18	0.8404

Time grooming, %	16	0.65	0.7249	18.5	0.35	0.7077	3.1 ± 1.20	0.6 ± 0.42	23.9	4.17	0.0524	23.9	1.56	0.2300
Time fighting, %	21.7	0.00	0.9998	23.9	0.00	0.9991	2.1 ± 0.00	0.1 ± 0.00	24	Infty	<.0001	24	0.00	0.9992
Time social grooming, %	16	0.98	0.4869	16	1.00	0.3904	0.0 ± 0.75	1.2 ± 0.75	24	1.19	0.2871	24	1.01	0.3807
Time sniffing other cow, %	16	0.87	0.5586	16	0.62	0.5516	0.0 ± 0.13	0.2 ± 0.13	24	1.90	0.1809	24	0.55	0.5837
Time sniffing environment, %	16	0.45	0.8719	16	0.13	0.8805	2.7 ± 0.85	2.4 ± 0.85	24	0.04	0.8376	24	0.74	0.4889
Time ingestion, %	16	1.18	0.3686	16	1.64	0.2247	**52.6 ± 7.56**	**29.7 ± 7.56**	24	4.57	**0.0428**	24	0.40	0.6763
Time social, %	16	0.77	0.6307	16	0.69	0.5163	2.2 ± 1.60	1.5 ± 1.60	24	0.09	0.7613	24	1.12	0.3437
Time exploration, %	16	0.45	0.8719	16	0.13	0.8805	2.7 ± 0.85	2.4 ± 0.85	24	0.04	0.8376	24	0.74	0.4889
Time active, %	16	1.22	0.3473	16	1.91	0.1805	57.5 ± 7.55	33.6 ± 7.55	24	5.42	0.0287	24	0.43	0.6559
Time inactive, %	16	1.12	0.3990	16	0.52	0.6052	**10.2 ± 6.48**	**30.1 ± 6.48**	24	4.44	**0.0456**	24	0.07	0.9302
Time eating, % ingestion	16	1.06	0.4336	16	0.06	0.9452	69.5 ± 9.52	44.4 ± 9.52	24	3.78	0.0638	24	0.64	0.5382
Time drinking, % ingestion	16	0.71	0.6803	21.5	0.72	0.4996	0.9 ± 0.66	3.7 ± 3.75	24	0.56	0.4596	24	1.22	0.3135
Time fighting, % social	16.1	1.40	0.2682	22	0.67	0.5224	14.8 ± 7.0	2.0 ± 1.81	24.2	2.84	0.1047	24.2	0.06	0.9396

Time social grooming, % social	16	0.82	0.5953	16	1.52	0.2488	0.0 ± 3.72	9.2 ± 3.72	24	3.13	0.0895	24	1.62	0.2197
Time sniffing other cow, % social	16	0.86	0.5696	16	0.57	0.5758	3.7 ± 4.63	7.4 ± 4.63	24	1.00	0.3273	24	1.00	0.3827
Time ingestion, % active	16	1.16	0.3806	16	0.14	0.8746	65.9 ± 8.74	41.7 ± 8.74	24	4.48	0.0450	24	0.41	0.6663
Time social, % active	16	0.68	0.7002	16	0.90	0.4266	3.7 ± 4.49	7.9 ± 4.49	24	0.42	0.5218	24	0.23	0.7982
Time exploration, % active	16	0.84	0.5788	16	0.40	0.6793	4.4 ± 1.90	6.0 ± 1.90	24	0.31	0.5821	24	0.15	0.8599
Time resting, % inactive	16	0.98	0.4849	16	0.27	0.7637	17.5 ± 8.62	37.0 ± 8.62	24	2.69	0.1143	24	0.63	0.5409
Time grooming, % inactive	16	0.75	0.6490	16	0.25	0.7784	34.4 ± 8.38	14.8 ± 8.38	24	5.10	0.0332	24	2.86	0.0768

[1] A significant time effect was found for the time spent in ingestion and inactive (P < 0.05). The time spent in ingestion decreased and the time spent inactive increased after one period of treatment application. These behaviors observed during the four minutes before the novel object test cannot be representative of a day. However, this could suggest that behaviors such as ingestion and inactivity were moved at another moment of the day. Our hypothesis is that the experiment, especially days of behavioral tests, was disturbing for animals, since we were asking them to stand often for procedures, in addition to the three outings of the other animals in the day. It is possible the cows would take advantage of any calm moment to be inactive.

Appendix Table 2. Estimated means (LSmeans ± SE) for frequencies (frq) and times spent performing different behaviors observed before the novel object test (model 2/objective 3) for time effect before and after overall experience and significance of time, group, sequence(Group), block(Group) and the interaction between sequence and time.[1]

Outcome measure	Group			Sequence(Group)			Block(Group)			Time					Sequence*Time		
	Ddf	F-value	P-value	Ddf	F-value	P-value	Ddf	F-value	P-value	Before	After	Ddf	F-value	P-value	Ddf	F-value	P-value
Frq eating, nb/min	14	0.55	0.4697	14	1.94	0.1594	14	0.84	0.5754	0.3 ± 0.05	0.2 ± 0.05	21	4.54	0.0451	21	0.40	0.8443
Frq drinking, nb/min	14	2.28	0.1531	14	0.48	0.7475	14	0.90	0.5312	0.0 ± 0.02	0.0 ± 0.02	21	0.62	0.4387	21	0.62	0.6852
Frq ruminating, nb/min	14	0.46	0.5086	14	0.64	0.6414	14	2.01	0.1258	0.0 ± 0.03	0.1 ± 0.03	21	1.28	0.2707	21	0.76	0.5886
Frq resting, nb/min	14	0.39	0.5423	14	0.65	0.6335	14	2.37	0.0802	0.1 ± 0.04	0.2 ± 0.04	21	3.72	0.0673	21	0.76	0.5880
Frq defecating, nb/min	14	5.53	0.0338	14	0.39	0.8131	14	2.67	0.0561	0.0 ± 0.01	0.0 ± 0.01	21	0.69	0.4151	21	0.29	0.9110
Frq urinating, nb/min	14	0.78	0.3927	14	0.87	0.5033	14	1.00	0.4706	0.0 ± 0.01	0.0 ± 0.01	21	0.78	0.3878	21	0.86	0.5266
Frq grooming, nb/min	14	0.56	0.4679	14	2.35	0.1042	14	0.43	0.8679	0.2 ± 0.05	0.1 ± 0.05	21	0.02	0.8951	21	0.57	0.7195
Frq fighting, nb/min	14	0.39	0.5416	14	2.14	0.1299	14	0.84	0.5735	0.1 ± 0.04	0.1 ± 0.04	21	0.19	0.6634	21	0.44	0.8172
Frq receiving fighting, nb/min	14	3.89	0.0687	14	1.17	0.3671	14	0.33	0.9255	0.0 ± 0.01	0.0 ± 0.01	21	5.00	0.0363	21	2.20	0.0928
Frq social grooming, nb/min	18.5	0.19	0.6706	18.5	1.21	0.3408	14	1.42	0.2720	0.0 ± 0.01	0.1 ± 0.04	21	1.40	0.2499	21	1.07	0.4052

Frq sniffing other cow, nb/min	14	1.41	0.2547	14	0.72	0.5917	14	1.36	0.2943	0.0 ± 0.03	0.0 ± 0.03	21	0.03	0.8700	21	0.73	0.6096
Frq sniffing environment, nb/min	14	0.97	0.3423	14	0.43	0.7853	14	0.81	0.5907	0.2 ± 0.05	0.1 ± 0.05	21	0.12	0.7374	21	0.94	0.4768
Frq standing, nb/min	14	0.01	0.9390	14	0.87	0.5033	14	1.49	0.2478	0.2 ± 0.02	0.1 ± 0.02	21	2.31	0.1431	21	0.14	0.9800
Frq lying, nb/min	14	0.12	0.7293	14	1.44	0.2737	14	0.87	0.5556	0.1 ± 0.02	0.1 ± 0.02	21	3.05	0.0953	21	0.32	0.8949
Time standing, %	14	0.01	0.9119	14	0.98	0.4513	14	0.88	0.5453	77.8 ± 9.40	54.3 ± 9.40	21	2.68	0.1165	21	0.36	0.8700
Time lying, %	14	0.01	0.9119	14	0.98	0.4513	14	0.88	0.5453	22.2 ± 9.40	45.7 ± 9.40	21	2.68	0.1165	21	0.36	0.8700
Time ruminating, %	14	0.50	0.4914	14	0.65	0.6361	14	3.81	0.0160	11.1 ± 6.59	27.6 ± 6.59	21	2.14	0.1585	21	1.05	0.4134
Time receiving fighting, %	14	1.30	0.2740	14	1.17	0.3671	14	1.00	0.4706	0.1 ± 0.04	0.0 ± 0.04	21	1.30	0.2677	21	1.19	0.3464
Time eating, %	14	0.26	0.6153	14	3.22	0.0452	14	1.55	0.2291	51.6 ± 7.01	23.0± 7.01	21	6.66	0.0174	21	0.40	0.8400
Time drinking, %	19.7	2.71	0.1155	19.7	0.44	0.7768	14	0.94	0.5062	0.8 ± 0.57	4.1 ± 2.26	21	1.94	0.1786	21	0.88	0.5106
Time resting, %	15.5	0.11	0.7493	15.5	0.80	0.5440	14.2	1.75	0.1746	6.8 ± 3.32	37.7 ± 8.86	21.5	8.66	0.0077	21.5	0.29	0.9118
Time grooming, %	14	2.45	0.1398	14	1.39	0.2877	14	0.38	0.9010	3.3 ± 1.00	1.8 ± 1.00	21	1.28	0.2702	21	0.78	0.5743
Time fighting, %	14	0.82	0.3794	14	1.10	0.3946	14	0.99	0.4788	2.0 ± 1.39	0.2 ± 1.39	21	0.79	0.3836	21	0.71	0.6229
Time social grooming, %	14	0.07	0.7914	14	1.04	0.4219	14	1.00	0.4706	0.0 ± 0.99	2.0 ± 0.99	21	2.07	0.1654	21	0.85	0.5328

Time sniffing other cow, %	14	0.60	0.4496	14	0.94	0.4672	14	2.52	0.0665	0.0 ± 0.05	0.1 ± 0.05	21	0.40	0.5333	21	0.97	0.4561
Time sniffing environment, %	14	1.54	0.2348	14	0.84	0.5215	14	0.94	0.5064	2.6 ± 0.74	1.6 ± 0.74	21	0.97	0.3362	21	1.54	0.2212
Time ingestion, %	14	0.01	0.9361	14	3.61	0.0320	14	1.93	0.1389	**52.4 ± 7.20**	**27.1 ± 7.20**	21	4.49	**0.0462**	21	0.28	0.9191
Time social, %	14	0.53	0.4774	14	1.26	0.3299	14	2.35	0.0825	2.0 ± 1.57	2.3 ± 1.57	21	0.01	0.9114	21	0.95	0.4694
Time exploration, %	14	1.54	0.2348	14	0.84	0.5215	14	0.94	0.5064	2.6 ± 0.74	1.6 ± 0.74	21	0.97	0.3362	21	1.54	0.2212
Time active, %	14	0.05	0.8185	14	3.02	0.0546	14	1.56	0.2264	57.0 ± 7.23	31.0 ± 7.23	21	5.22	0.0328	21	0.57	0.7243
Time inactive, %	14	0.37	0.5501	14	0.78	0.5584	14	1.43	0.2686	**10.1 ± 6.57**	**39.5 ± 6.57**	21	8.24	**0.0092**	21	0.25	0.9327
Time eating, % ingestion	14	0.01	0.9165	14	2.81	0.0665	14	1.80	0.1662	69.1 ± 8.20	30.8 ± 8.20	21	9.40	0.0059	21	0.45	0.8081
Time drinking, % ingestion	21	2.95	0.1005	21	0.09	0.9840	14	0.96	0.4931	0.9 ± 0.63	9.2 ± 5.19	21.3	2.44	0.1333	21.3	0.69	0.6395
Time fighting, % social	14	0.39	0.5429	14	2.33	0.1064	14	0.50	0.8195	14.2 ± 6.41	7.5 ± 6.41	21	0.44	0.5122	21	0.19	0.9635
Time social grooming, % social	14	0.03	0.8764	14	1.02	0.4324	14	1.00	0.4706	0.0 ± 3.72	7.5 ± 3.72	21	2.03	0.1687	21	0.82	0.5505
Time sniffing other cow, % social	14	0.23	0.6420	14	1.02	0.4324	14	2.55	0.0646	4.2 ± 4.17	6.7 ± 4.17	21	0.15	0.7034	21	1.10	0.3921
Time ingestion, % active	14	0.64	0.4368	14	3.44	0.0370	14	2.31	0.0862	65.7 ± 8.40	37.6 ± 8.40	21	3.97	0.0594	21	0.27	0.9252
Time social, % active	14	0.87	0.3657	14	1.49	0.2567	14	1.80	0.1658	3.4 ± 4.93	11.1 ± 4.93	21	0.99	0.3318	21	0.35	0.8788

Time exploration, % active	14	3.62	0.0778	14	2.33	0.1064	14	1.37	0.2902	4.2 ± 2.69	5.4 ± 2.69	21	0.08	0.7851	21	0.96	0.4636
Time resting, % inactive	14	0.00	0.9458	14	1.09	0.4006	14	2.27	0.0909	17.2 ± 8.39	47.9 ± 8.39	21	5.30	0.0317	21	0.47	0.7958
Time grooming, % inactive	14	1.10	0.3113	14	1.97	0.1547	14	0.42	0.8711	35.3 ± 8.59	19.6 ± 8.59	21	2.11	0.1610	21	0.56	0.7280

[1] A significant time effect was found for the time spent in ingestion and inactive ($P < 0.05$). The time spent in ingestion decreased and the time spent inactive increased after the overall experience. This cannot be representative of their whole day schedule. However, this test was performed at three different moments in the day (morning, lunchtime, afternoon). As for after one period of treatment application, we supposed the experiment (outings and/or tests) were disturbing for the animals, since we asked them to stand often. Consequently, we supposed the animals would take advantage of any time off to be inactive. Cows might also have redistributed their activities such as ingestion towards other moments in the day.

Appendix Table 3. Matrix compiling the variances (diagonal), co-variances (above the diagonal) and correlations (below the diagonal) for before and after first period of treatment application for the frequency of social grooming before the novel object test (model 1/objective 2)

	After	**Before**
After	0.0135	0.0000
Before	0.0000	0.0022

Appendix Table 4. Matrix compiling the variances (diagonal), co-variances (above the diagonal) and correlations (below the diagonal) for before and after first period of treatment application for the time spent receiving fighting before the novel object test (model 1/objective 2)

	After	**Before**
After	27.0311	0.0148
Before	0.0312	0.0083

Appendix Table 5. Matrix compiling the variances (diagonal), co-variances (above the diagonal) and correlations (below the diagonal) for before and after first period of treatment application for the time spent drinking before the novel object test (model 1/objective 2)

	After	**Before**
After	1.3577	0.0072
Before	0.0020	9.3819

Appendix Table 6. Matrix compiling the variances (diagonal), co-variances (above the diagonal) and correlations (below the diagonal) for before and after first period of treatment application for the time spent resting before the novel object test (model 1/objective 2)

	After	**Before**
After	2127.4100	-4.1429
Before	-0.0056	259.4500

Appendix Table 7. Matrix compiling the variances (diagonal), co-variances (above the diagonal) and correlations (below the diagonal) for before and after first period of treatment application for the time spent grooming before the novel object test (model 1/objective 2)

	After	**Before**
After	2.2343	-0.0447
Before	-0.0050	36.2136

Appendix Table 8. Matrix compiling the variances (diagonal), co-variances (above the diagonal) and correlations (below the diagonal) for before and after first period of treatment application for the time spent fighting before the novel object test (model 1/objective 2)

	After	**Before**
After	0.1315	-0.4310
Before	-0.1186	100.4600

Appendix Table 9. Matrix compiling the variances (diagonal), co-variances (above the diagonal) and correlations (below the diagonal) for before and after first period of treatment application for the time spent drinking (% ingestion) before the novel object test (model 1/objective 2)

	After	**Before**
After	375.6500	0.6180
Before	0.0121	7.0001

Appendix Table 10. Matrix compiling the variances (diagonal), co-variances (above the diagonal) and correlations (below the diagonal) for before and after first period of treatment application for the time spent fighting (% social) before the novel object test (model 1/objective 2)

	After	**Before**
After	21.0490	-154.2700
Before	-0.9542	1241.9200

Appendix Table 11. Matrix compiling the variances (diagonal), co-variances (above the diagonal) and correlations (below the diagonal) for before and after first period of treatment application for the latency to approach during the novel object test (model 1/objective 2)

	After	**Before**
After	3042.4900	53.2440
Before	0.1859	26.9606

Appendix Table 12. Matrix compiling the variances (diagonal), co-variances (above the diagonal) and correlations (below the diagonal) for before and after first period of treatment application for the latency to move away during the novel object test (model 1/objective 2)

	After	**Before**
After	173.5100	-9.4358
Before	-0.1378	27.0327

Appendix Table 13. Matrix compiling the variances (diagonal), co-variances (above the diagonal) and correlations (below the diagonal) for before and after first period of treatment application for the time spent licking during the novel object test (model 1/objective 2)

	After	**Before**
After	5.1836	-8.2524
Before	-0.4119	77.4309

Appendix Table 14. Matrix compiling the variances (diagonal), co-variances (above the diagonal) and correlations (below the diagonal) for before and after first period of treatment application for the time spent resting during the novel object test (model 1/objective 2)

	After	**Before**
After	882.4500	-77.6672
Before	-0.3700	49.9233

Appendix Table 15. Matrix compiling the variances (diagonal), co-variances (above the diagonal) and correlations (below the diagonal) for before and after first period of treatment application for the time spent at < 1 head (% distance) during the novel object test (model 1/objective 2)

	After	**Before**
After	414.6000	16.5911
Before	0.6307	1.6693

Appendix Table 16. Matrix compiling the variances (diagonal), co-variances (above the diagonal) and correlations (below the diagonal) for before and after the overall experience for the stage 1 during human test (model 2/objective 3)

	After	**Before**
After	0.0026	0.0000
Before	0.0000	0.0704

Appendix Table 17. Matrix compiling the variances (diagonal), co-variances (above the diagonal) and correlations (below the diagonal) for before and after the overall experience for the stage 5 during human test (model 2/objective 3)

	After	**Before**
After	2.0537	0.1689
Before	0.4881	0.0583

Appendix Table 18. Matrix compiling the variances (diagonal), co-variances (above the diagonal) and correlations (below the diagonal) for before and after the overall experience for the mean during human test (model 2/objective 3)

	After	**Before**
After	0.2426	0.0004
Before	0.0063	0.0196

Appendix Table 19. Matrix compiling the variances (diagonal), co-variances (above the diagonal) and correlations (below the diagonal) for before and after the overall experience for the frequency of social grooming before the novel object test (model 2/objective 3)

	After	**Before**
After	0.0413	-0.0020
Before	-0.2023	0.0023

Appendix Table 20. Matrix compiling the variances (diagonal), co-variances (above the diagonal) and correlations (below the diagonal) for before and after the overall experience for the time spent drinking before the novel object test (model 2/objective 3)

	After	**Before**
After	130.4600	-9.0138
Before	-0.4867	2.6296

Appendix Table 21. Matrix compiling the variances (diagonal), co-variances (above the diagonal) and correlations (below the diagonal) for before and after the overall experience for the time spent resting before the novel object test (model 2/objective 3)

	After	Before
After	2087.3500	-289.6600
Before	-0.3753	285.3400

Appendix Table 22. Matrix compiling the variances (diagonal), co-variances (above the diagonal) and correlations (below the diagonal) for before and after the overall experience for the time spent drinking (% ingestion) before the novel object test (model 2/objective 3)

	After	Before
After	710.2000	-18.5721
Before	-0.5049	1.9054

Appendix Table 23. Matrix compiling the variances (diagonal), co-variances (above the diagonal) and correlations (below the diagonal) for before and after the overall experience for the time spent resting during the novel object test (model 2/objective 3)

	After	Before
After	1361.2700	-28.6157
Before	-0.0802	93.4276

Appendix Table 24. Matrix compiling the variances (diagonal), co-variances (above the diagonal) and correlations (below the diagonal) for before and after the overall experience for the frequency maximum distance during the novel object test (model 2/objective 3)

	After	**Before**
After	0.0257	0.0013
Before	0.0118	0.4649

Appendix Table 25. Matrix compiling the variances (diagonal), co-variances (above the diagonal) and correlations (below the diagonal) for before and after the overall experience for the time spent at < 1 head (% distance) distance during the novel object test (model 2/objective 3)

	After	**Before**
After	233.8700	0.7660
Before	0.0096	27.0529

Appendix Table 26. Random effects variances (σ^2_{cow}, σ^2_e, CS), covariance parameter estimates, phenotypic variance (σ^2_p)[1], variable means ($\overline{x}$)[2], and coefficient of variation (CV)[3] for the time to put on the halter, trip speed and total speed in the trip test (objective 1)

Outcome measure	σ^2_{cow}	AR(1)	CS	σ^2_e	σ^2_p	$\overline{x}$	CV (%)
Time to put on the halter, s	-	-	39.08	88.44	127.52	-0.18	-6265.73
Trip speed, m/s	-	-	0.12	0.20	0.32	0.02	3398.22
Total speed, m/s	-	-	0.00	0.01	0.01	0.00	11074.96

[1] $\sigma^2_p = \sigma^2_{cow} + \sigma^2_e$
[2] $\overline{x}$ = the average between the two treatment LSmeans
[3] CV = (sqrt (σ^2_p) / $\overline{x}$) * 100

Appendix Table 27. Random effects variances (σ^2_{cow}, σ^2_e, CS), covariance parameter estimates, phenotypic variance (σ^2_p)[1], variable means ($\overline{x}$)[2], and coefficient of variation (CV)[3] for the latency, stages 1 to 5 and average of stages (mean) in the human test from model 1 (objective 2)

Outcome measure	σ^2_{cow}	AR(1)	CS	σ^2_e	σ^2_p	$\overline{x}$	CV (%)
Latency, s	-	-	4.92	10.31	15.23	22.75	0.45
Stage 1	-	-	-0.00	0.04	0.04	-0.02	-778.13
Stage 2	-	-	0.04	0.12	0.16	-0.13	-318.94
Stage 3	-	-	0.76	0.66	1.42	0.10	1136.93
Stage 4	-	-	0.84	0.38	1.22	-0.34	-327.68
Stage 5	-	-	0.18	0.41	0.59	-1.64	-46.89
Mean	-	-	0.18	0.09	0.27	-0.40	-128.84

[1] $\sigma^2_p = \sigma^2_{cow} + \sigma^2_e$
[2] $\overline{x}$ = the average between the two treatment LSmeans
[3] CV = (sqrt (σ^2_p) / $\overline{x}$) * 100

Appendix Table 28. Random effects variances (σ^2_{cow}, σ^2_e, CS), covariance parameter estimates, phenotypic variance (σ^2_p)[1], variable means ($\overline{x}$)[2], and coefficient of variation (CV)[3] for the reaction and freezing time in the suddenness test from model 1 (objective 2)

Outcome measure	σ^2_{cow}	AR(1)	CS	σ^2_e	σ^2_p	$\overline{x}$	CV (%)
Reaction	-	-	0.43	0.55	0.98	1.28	77.62
Freezing time, s	-	-	55.11	57.33	112.43	13.93	76.14

[1] $\sigma^2_p = \sigma^2_{cow} + \sigma^2_e$
[2] $\overline{x}$ = the average between the two treatment LSmeans
[3] CV = (sqrt (σ^2_p) / $\overline{x}$) * 100

Appendix Table 29. Random effects variances (σ^2_{cow}, σ^2_e, CS), covariance parameter estimates, phenotypic variance (σ^2_p)[1], variable means ($\bar{x}$)[2], and coefficient of variation (CV)[3] for frequencies (frq) and times spent in different behaviors observed before the novel object test from model 1 (objective 2)

Outcome measure	σ^2_{cow}	AR(1)	CS	σ^2_e	σ^2_p	$\bar{x}$	CV (%)
Frq eating, nb/min	-	-	0.00	0.09	0.09	0.26	114.24
Frq drinking, nb/min	-	-	0.00	0.00	0.00	0.01	437.27
Frq ruminating, nb/min	-	-	0.00	0.03	0.02	0.08	184.32
Frq resting, nb/min	-	-	0.00	0.03	0.03	0.10	169.17
Frq defecating, nb/min	-	-	0.00	0.01	0.01	0.02	326.58
Frq urinating, nb/min	-	-	0.00	0.00	0.00	0.00	734.66
Frq grooming, nb/min	-	-	0.01	0.03	0.04	0.10	200.15
Frq fighting, nb/min	-	-	0.00	0.02	0.01	0.06	217.03
Frq receiving fighting, nb/min	-	-	0.00	0.01	0.01	0.03	338.14
Frq sniffing other cow, nb/min	-	-	0.00	0.01	0.01	0.03	387.27
Frq sniffing environment, nb/min	-	-	-0.01	0.07	0.06	0.17	142.31
Frq standing, nb/min	-	-	0.00	0.01	0.01	0.19	56.52
Frq lying, nb/min	-	-	0.00	0.01	0.01	0.07	152.79
Time standing, %	-	-	256.38	1548.20	1804.58	73.29	57.96
Time lying, %	-	-	256.38	1548.20	1804.58	26.71	159.03
Time ruminating, %	-	-	93.96	1359.36	1453.32	23.97	159.02
Time eating, %	-	-	-28.30	1561.70	1533.40	40.68	96.26
Time social grooming, %	-	-	0.06	15.26	15.32	0.58	676.29
Time sniffing other cow, %	-	-	0.14	0.29	0.43	0.13	497.94
Time sniffing environment, %	-	-	-1.94	21.64	19.70	2.55	173.80
Time ingestion, %	-	-	-4.34	1548.00	1543.66	41.19	95.39
Time social, %	-	-	0.35	68.96	69.31	1.83	455.26
Time exploration, %	-	-	-1.94	21.64	19.70	2.55	173.80
Time active, %	-	-	121.76	1418.97	1540.73	45.57	86.13
Time inactive, %	-	-	-74.68	1209.34	1134.66	20.13	167.33

Time eating, % ingestion	-	-	201.77	2242.87	2444.64	56.97	86.79
Time social grooming, % social	-	-	11.41	361.39	372.80	4.58	421.80
Time sniffing other cow, % social	-	-	393.52	185.19	578.71	5.56	433.01
Time ingestion, % active	-	-	293.24	1771.06	2064.30	53.81	84.44
Time social, % active	-	-	-16.13	561.10	544.97	5.81	401.67
Time exploration, % active	-	-	-2.62	100.24	97.62	5.20	190.09
Time resting, % inactive	-	-	85.29	1920.78	2006.07	27.26	164.28
Time grooming, % inactive	-	-	884.01	1010.52	1894.53	24.59	177.02

[1] $\sigma^2_p = \sigma^2_{cow} + \sigma^2_e$

[2] $\overline{x}$ = the average between the two treatment LSmeans

[3] CV = (sqrt (σ^2_p) / $\overline{x}$) * 100

Appendix Table 30. Random effects variances (σ^2_{cow}, σ^2_e, CS), covariance parameter estimates, phenotypic variance (σ^2_p)[1], variable means ($\bar{x}$)[2], and coefficient of variation (CV)[3] for latencies, frequencies (frq) and times spent in different behaviors observed during the novel object test from model 1 (objective 2)

Outcome measure	σ^2_{cow}	AR(1)	CS	σ^2_e	σ^2_p	$\bar{x}$	CV (%)
Latency sniff, s	-	-	-480.62	13429.00	12948.38	93.04	122.31
Latency lick, s	-	-	298.84	5981.14	6279.98	249.02	31.82
Latency push, s	-	-	2569.41	4524.89	7094.30	250.33	33.65
Frq look, nb/min	-	-	0.06	0.16	0.22	1.15	40.79
Frq approach, nb/min	-	-	0.16	0.30	0.46	1.44	47.21
Frq move away, nb/min	-	-	0.10	0.33	0.43	1.42	46.08
Frq sniff, nb/min	-	-	-0.05	0.28	0.24	0.49	99.54
Frq lick, nb/min	-	-	-0.01	0.11	0.10	0.16	195.49
Frq push, nb/min	-	-	0.03	0.06	0.08	0.14	204.43
Time look, %	-	-	10.50	340.68	351.18	31.30	59.87
Time interaction, %	-	-	-12.48	95.28	82.80	7.58	120.04
Time sniff, %	-	-	-3.68	22.16	18.48	3.06	140.69
Time push, %	-	-	-0.68	37.50	36.82	2.44	248.87
Time sniff, % interaction	-	-	670.36	1632.33	2302.69	56.79	84.50
Time lick, % interaction	-	-	-43.77	379.60	335.83	7.48	244.96
Time push, % interaction	-	-	250.94	494.58	745.52	11.66	234.24
Frq eating, nb/min	-	-	0.00	0.04	0.04	0.18	108.26
Frq drinking, nb/min	-	-	0.00	0.00	0.01	0.02	399.09
Frq ruminating, nb/min	-	-	0.00	0.06	0.06	0.16	160.61
Frq resting, nb/min	-	-	0.01	0.02	0.03	0.08	200.78
Time ruminating, %	-	-	38.96	846.86	885.82	20.69	143.84
Time eating, %	-	-	-26.51	192.47	165.96	10.96	117.58
Time drinking, %	-	-	8.95	38.99	47.94	1.35	514.50
Frq touch, nb/min	-	-	0.14	0.68	0.82	0.97	93.60
Frq < 1 head, nb/min	-	-	0.30	2.26	2.56	2.24	71.38
Frq 1 to 2 heads, nb/min	-	-	0.00	1.83	1.82	2.61	51.80

Frq > 2 heads, nb/min	-	-	0.51	0.71	1.22	1.93	57.24
Frq maximum, nb/min	-	-	0.25	0.33	0.58	0.51	147.52
Time touch, % distance	-	-	-5.57	138.91	133.34	8.93	129.28
Time 1 to 2 heads, % distance	-	-	-62.97	340.44	277.47	14.28	116.63
Time > 2 heads, % distance	-	-	16.37	848.33	864.70	52.95	55.54
Time maximum, % distance	-	-	152.59	263.94	416.53	13.61	149.93

[1] $\sigma^2_p = \sigma^2_{cow} + \sigma^2_e$

[2] $\overline{x}$ = the average between the two treatment LSmeans

[3] CV = (sqrt (σ^2_p) / $\overline{x}$) * 100

Appendix Table 31. Random effects variances (σ^2_{cow}, σ^2_e, CS), covariance parameter estimates, phenotypic variance (σ^2_p)[1], variable means ($\overline{x}$)[2], and coefficient of variation (CV)[3] for the latency and stages 2 to 4 in the human test from model 2 (objective 3)

Outcome measure	σ^2_{cow}	AR(1)	CS	σ^2_e	σ^2_p	$\overline{x}$	CV (%)
Latency, s	-	-	9.86	8.49	18.34	21.93	19.53
Stage 2	-	-	0.11	0.09	0.20	-0.05	-826.34
Stage 3	-	-	1.08	0.58	1.66	0.21	600.32
Stage 4	-	-	0.99	0.61	1.61	-0.10	-1267.12

1 $\sigma^2_p = \sigma^2_{cow} + \sigma^2_e$
2 $\overline{x}$ = the average between the two treatment LSmeans
3 CV = (sqrt (σ^2_p) / $\overline{x}$) * 100

Appendix Table 32. Random effects variances (σ^2_{cow}, σ^2_e, CS), covariance parameter estimates, phenotypic variance (σ^2_p)[1], variable means ($\overline{x}$)[2], and coefficient of variation (CV)[3] for the reaction and freezing time in the suddenness test from model 2 (objective 3)

Outcome measure	σ^2_{cow}	AR(1)	CS	σ^2_e	σ^2_p	$\overline{x}$	CV (%)
Reaction	-	-	0.44	0.53	0.97	1.17	84.48
Freezing time, s	-	-	52.26	76.36	128.62	13.75	82.48

1 $\sigma^2_p = \sigma^2_{cow} + \sigma^2_e$
2 $\overline{x}$ = the average between the two treatment LSmeans
3 CV = (sqrt (σ^2_p) / $\overline{x}$) * 100

Appendix Table 33. Random effects variances (σ^2_{cow}, σ^2_e, CS), covariance parameter estimates, phenotypic variance (σ^2_p)[1], variable means ($\overline{x}$)[2], and coefficient of variation (CV)[3] for frequencies (frq) and times spent in different behaviors observed before the novel object test from model 2 (objective 3)

Outcome measure	σ^2_{cow}	AR(1)	CS	σ^2_e	σ^2_p	$\overline{x}$	CV (%)
Frq eating, nb/min	-	-	-0.01	0.08	0.08	0.24	113.26
Frq drinking, nb/min	-	-	0.00	0.01	0.01	0.03	334.31
Frq ruminating, nb/min	-	-	-0.01	0.03	0.02	0.07	190.57
Frq resting, nb/min	-	-	-0.01	0.04	0.03	0.13	144.75
Frq defecating, nb/min	-	-	0.00	0.01	0.00	0.02	380.20
Frq urinating, nb/min	-	-	0.00	0.00	0.00	0.00	827.85
Frq grooming, nb/min	-	-	-0.01	0.08	0.07	0.15	179.02
Frq fighting, nb/min	-	-	0.00	0.04	0.03	0.07	274.72
Frq receiving fighting, nb/min	-	-	0.00	0.00	0.00	0.02	349.25
Frq sniffing other cow, nb/min	-	-	0.00	0.02	0.02	0.03	430.19
Frq sniffing environment, nb/min	-	-	-0.01	0.06	0.06	0.14	162.71
Frq standing, nb/min	-	-	0.00	0.02	0.01	0.18	66.94
Frq lying, nb/min	-	-	0.00	0.02	0.01	0.09	137.96
Time standing, %	-	-	-401.96	2759.52	2357.56	66.04	73.52
Time lying, %	-	-	-401.96	2759.52	2357.56	33.96	142.98
Time ruminating, %	-	-	-533.55	1690.98	1157.43	19.36	175.72
Time receiving fighting, %	-	-	0.00	0.05	0.05	0.03	641.45
Time eating, %	-	-	-325.46	1635.41	1309.95	37.31	97.02
Time grooming, %	-	-	2.80	23.93	26.73	2.57	201.36
Time fighting, %			-2.04	53.84	51.80	1.07	674.09
Time social grooming, %	-	-	0.00	26.27	26.27	1.01	508.13
Time sniffing other cow, %	-	-	-0.01	0.08	0.07	0.06	432.93
Time sniffing environment, %	-	-	0.45	14.24	14.69	2.10	182.31
Time ingestion, %	-	-	-513.53	1897.46	1383.93	39.77	93.53
Time social, %	-	-	-15.25	81.19	65.94	2.14	380.28

Time exploration, %	-	-	0.45	14.24	14.69	2.10	182.31
Time active, %	-	-	-333.48	1728.57	1395.09	44.01	84.86
Time inactive, %	-	-	-253.07	1402.55	1149.48	24.82	136.62
Time eating, % ingestion	-	-	-285.97	2078.75	1792.78	49.95	84.77
Time fighting, % social	-	-	-238.10	1333.33	1095.23	10.83	305.49
Time social grooming, % social	-	-	0.00	369.05	369.05	3.75	512.28
Time sniffing other cow, % social	-	-	-95.24	559.52	464.28	5.42	397.79
Time ingestion, % active	-	-	-768.30	2650.25	1881.95	51.66	83.98
Time social, % active	-	-	-164.34	811.72	647.38	7.27	349.94
Time exploration, % active	-	-	-51.10	244.74	193.64	4.82	288.73
Time resting, % inactive	-	-	-494.75	2372.18	1877.43	32.53	133.20
Time grooming, % inactive	-	-	411.65	1557.52	1969.17	27.47	161.53

[1] $\sigma^2_p = \sigma^2_{cow} + \sigma^2_e$

[2] $\overline{x}$ = the average between the two treatment LSmeans

[3] CV = (sqrt (σ^2_p) / $\overline{x}$) * 100

Appendix Table 34. Random effects variances (σ^2_{cow}, σ^2_e, CS), covariance parameter estimates, phenotypic variance (σ^2_p)[1], variable means ($\overline{x}$)[2], and coefficient of variation (CV)[3] for latencies, frequencies (frq) and times spent in different behaviors observed during the novel object test from model 2 (objective 3)

Outcome measure	σ^2_{cow}	AR(1)	CS	σ^2_e	σ^2_p	$\overline{x}$	CV (%)
Latency approach, s	-	-	2.33	82.44	84.77	11.13	82.70
Latency move away, s	-	-	8.19	82.45	90.63	15.52	61.35
Latency sniff, s	-	-	667.10	8958.54	9625.64	69.23	141.72
Latency lick, s	-	-	2837.78	5843.26	8681.04	238.62	39.05
Latency push, s	-	-	5949.44	4817.03	10766.47	240.95	43.06
Frq look, nb/min	-	-	-0.03	0.17	0.14	1.07	34.86
Frq approach, nb/min	-	-	-0.13	0.68	0.55	1.49	50.04
Frq move away, nb/min	-	-	-0.16	0.59	0.43	1.44	45.65
Frq sniff, nb/min	-	-	0.00	0.26	0.26	0.58	87.70
Frq lick, nb/min	-	-	0.05	0.10	0.16	0.21	185.36
Frq push, nb/min	-	-	0.06	0.26	0.32	0.23	249.73
Time look, %	-	-	-36.47	665.73	629.26	35.69	70.28
Time interaction, %	-	-	36.39	232.61	269.00	10.97	149.54
Time sniff, %	-	-	-0.08	22.87	22.78	3.65	130.83
Time lick, %			16.15	131.94	148.09	3.80	320.29
Time push, %	-	-	-5.61	86.46	80.84	3.52	255.43
Time sniff, % interaction	-	-	741.66	1238.48	1980.14	59.99	74.18
Time lick, % interaction	-	-	192.31	208.97	401.28	8.36	239.73
Time push, % interaction	-	-	273.82	611.55	885.37	14.57	204.21
Frq eating, nb/min	-	-	-0.02	0.10	0.09	0.25	119.44
Frq drinking, nb/min	-	-	0.00	0.00	0.00	0.01	512.25
Frq ruminating, nb/min	-	-	-0.03	0.10	0.07	0.13	203.93
Frq resting, nb/min	-	-	0.00	0.04	0.04	0.10	185.45
Time ruminating, %	-	-	-380.37	1312.72	932.35	15.90	191.99
Time eating, %	-	-	136.56	357.44	494.00	16.99	130.81
Time drinking, %	-	-	0.00	9.32	9.32	0.54	562.17

Frq touch, nb/min	-	-	0.35	0.60	0.95	1.15	84.57
Frq < 1 head, nb/min	-	-	0.89	2.23	3.12	2.50	70.66
Frq 1 to 2 heads, nb/min	-	-	-0.02	2.07	2.05	2.79	51.39
Frq > 2 heads, nb/min	-	-	-0.25	1.12	0.87	1.88	49.56
Time touch, % distance	-	-	84.79	264.90	349.69	12.37	151.14
Time 1 to 2 heads, % distance	-	-	156.92	266.18	423.10	15.72	130.81
Time > 2 heads, % distance	-	-	-37.90	750.22	712.32	53.03	50.33
Time maximum, % distance	-	-	2.61	398.98	401.59	10.30	194.49

[1] $\sigma^2_p = \sigma^2_{cow} + \sigma^2_e$

[2] $\overline{x}$ = the average between the two treatment LSmeans

[3] CV = (sqrt (σ^2_p) / $\overline{x}$) * 100