



Animal discomfort: A concept analysis using the domesticated pig (*Sus scrofa*) as a model

Guilherme A. Franchi^{a,*}, Marc Bagaria^b, Heleen Boswijk^c, Emma Fàbrega^b, Mette S. Herskin^a, Rebecka Westin^d

^a Aarhus University, Department of Animal and Veterinary Sciences, Blichers Allé 20, 8830 Tjele, Denmark

^b Institute of Agricultural Research and Technology, Animal Welfare Program, 17121 Monells, Spain

^c Wageningen University and Research, Real Estate and Housing, Facilities and Services, Akkermaalsbos 12, 6708 WB Wageningen, the Netherlands

^d Swedish University of Agricultural Sciences, Department of Applied Animal Science and Welfare, Box 234, 532 23 Skara, Sweden

HIGHLIGHTS

- The concept "animal discomfort" still remains unclear in literature.
- We performed a Walker and Avant concept analysis to define animal discomfort.
- Animal discomfort intersects on three domains: physical, physiological, and mental.
- Discomfort features a sense of uneasiness resulting in avoidance attempts.

ARTICLE INFO

Keywords:

Animal welfare
Affective state
Biomedical research
Intensive systems
Agricultural green transition

ABSTRACT

The term discomfort is frequently used in for example biomedical studies, animal experimentation for farming purposes, animal welfare legislation, and ethical permits for animal experimentation. However, the concept of "animal discomfort" still remains unclear. Using the domesticated pig as a model, we performed a Walker and Avant concept analysis to develop an operational definition of animal discomfort. A total of 2,594 documents published in English were retrieved from Scopus database. Among them, 118 were retained for analysis as they contained either: 1 - a definition and/or measurement of discomfort in animals, including pigs; 2 - definition and/or measurement of pain, suffering, or sickness in pigs only. The literature review showed that animal discomfort intersects on three domains: physical, physiological, and mental discomfort. The presence of discomfort leads to a sense of uneasiness that results in behaviorally visible consequences comprising animals' attempts to avoid or alleviate the source(s) of this affective state. Accordingly, our proposed operational definition of animal discomfort is: short- or long-lived negative affective state featured by physical, physiological and/or mental components, induced by internal or external stimuli, ranging from mild to severe, potentially occurring together with other negative affective states, and leading to avoidance or attempt to alleviate the source of uneasiness. Access to a shared definition of this central concept in animal welfare may be one initial step to facilitate legislation consistency, improve animal research integrity, and ultimately promote a more sustainable livestock production.

1. Introduction

In recent years, the biomedical community has increasingly recognized the relevance of employing pigs (*Sus scrofa*) in research endeavors. Pigs have for example proven to be valuable assets across diverse domains, encompassing the provision of organs for critical human

transplantation, enhancement of insights into various diseases, and serving as effective models for surgical training (EARA, 2023). Moreover, the homology between porcine and human attributes, including anatomical structure, organ systems, and genetics, renders pigs as advantageous candidates for investigating and optimizing scientific methodologies, as well as evaluating drug efficacy and safety, within

* Corresponding author.

E-mail address: gaf@anivet.au.dk (G.A. Franchi).

<https://doi.org/10.1016/j.livsci.2024.105524>

Received 20 February 2024; Received in revised form 5 July 2024; Accepted 8 July 2024

Available online 8 July 2024

1871-1413/© 2024 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

laboratory settings as well as clinical contexts (Almond, 1996; Douglas, 1972). Among the domesticated animals, pigs are special, because they are used for several purposes – as an animal model as well as for production of animal-origins protein. In 2019, a total of 82,819 pigs were subjected to scientific research within the European Union (EU) and approximately 143 million pigs were raised for meat production in the EU (EARA, 2023; Augère-Granier, 2020). The latter highlights the relevance and need for agricultural-focused research to commercial pork production. For instance, in pursuit of alternative feed sources for the animals, not suitable for human consumption, aiming to make pig production more sustainable (van der Heide et al., 2021), a substantial amount of resources (e.g., pigs, labor, time) and infrastructure (e.g., experimental facilities, balance chambers to quantify methane emissions – e.g., Friend and MacIntyre, 1969; Eskildsen et al., 2020) have been invested. Thus, over all the use of pigs for experimentation will likely not decrease in coming years.

Yet, when used in either biomedical or agricultural experimentation, pigs are often kept in environments featured by limited space and stimuli (e.g., few or no companions, no enrichment), thus, preventing them from performing natural and highly motivated behaviors such as exploration and socialization (as reviewed by Herskin et al., 2020). This imposed lack of behavioral expression has been associated with impoverished welfare (Dawkins, 1988). Additionally, roughly 98 % of pigs raised for pork production in the EU originate from farms following conventional husbandry practices (Augère-Granier, 2020), typically intensive and barren, which do not meet basic needs of pigs concerning fulfillment of motivations and cognitive stimulation (e.g., Murphy et al., 2014). Consequently, also conventionally reared pigs kept for meat production likely experience negative emotions and poor welfare. Herein, emotions are defined as brief yet intense reactions to a specific context, involving changes in conscious experience, behavior, cognition, and neurophysiology (Paul et al., 2005; Paul and Mendl, 2018). Emotions encompass two primary components: valence, which pertains to the attractiveness (positive valence) or aversiveness (negative valence) of a stimulus or context, and arousal, indicating the intensity (ranging from low to high) (Mendl et al., 2010; Russel, 2003). In this study, valenced states are referred to as "affective states," a comprehensive term often used interchangeably with emotional states, encompassing both short-term emotions and long-term moods (Murphy et al., 2021).

One negative affective state often mentioned when animal welfare is discussed or assessed is discomfort (Broom, 1998; Mellor, 2017). Freedom from discomfort constitutes one of the fundamental prerequisites for animal welfare, as outlined in the Five Freedoms and Five Welfare Provisions paradigms, which have played a pivotal role in shaping animal welfare legislation and policy (Mellor, 2016). Furthermore, the term "discomfort" is frequently employed in animal research focused on conditions or procedures eliciting negative affective states. As mentioned by Herskin et al. (2018), "animal discomfort" has been used and interpreted in several ways: for example involving mild pain, itchiness, breathlessness or nausea – all states that are relevant to animal experimentation and production. However, despite its widespread usage in science, legislation and policy-making, a universally accepted concept of "animal discomfort" still remains absent. Guesgen and Bench (2017) pointed out that discomfort as affective state in animals has simply been introduced as lack of comfort or interchangeably with a range of negative affective states such as fear, anxiety or milder forms of pain. Moreover, Guesgen and Bench (2017) stated that discomfort is often referred to separately from the term "distress", which suggests a more severe state, although is arguably vaguely defined as well. We suggest that the use of the term "animal discomfort" in animal research and legislation, and concomitant absence of or unclear conceptualization of this term may hamper the understanding of how pigs, as well as other domesticated animals, respond to different management practices and the potential affective consequences for the animals. Simultaneously, the lack of a definition of the state of discomfort means that an accurate selection of tools to objectively quantify the effects arising from the

presence of this negative affective state is, as a result, impeded. Thus, thorough assessment and understanding of the impact of management practices on affective aspects of welfare of pigs kept for meat production as well as research purposes can be challenged. Better understanding of the concept of discomfort can be one initial step to facilitate legislation consistency, improve animal research integrity, and ultimately promote a more sustainable livestock production.

Accordingly, the aim of this study was to conceptualize an operational definition of "animal discomfort" using domesticated pigs as a model. Concept analysis involves the examination of a concept's attributes and criteria to determine its exemplification, facilitating effective communication, theory advancement, and research endeavors (Ashkenazy and Ganz, 2019). We used the Walker and Avant (2005) concept analysis method because of its straightforward and systematic approach. Based on literature, we identified the uses of the concept, constructed a model case, and defined antecedents, consequences and empirical referents.

2. Materials and methods

This study was conducted from June 2023 to February 2024. A concept analysis for "animal discomfort" was conducted by searching the scientific literature using the Scopus database for full-text documents in English (i.e., scientific manuscripts, book chapters, and reviews) published until June 2023. Initially 2594 publications from animal and human literature were found using the keywords "ANIMAL" and "DISCOMFORT" and "DEFIN*". The list of publications was split equally among three trained experimenters (i.e., the first three authors of this study) who fully evaluated each publication using the following inclusion criteria: 1. Discomfort defined and/or measured in animals only; 2. Pain, suffering, or sickness defined and/or measured in pigs only. Among them, 118 (4.5 %) publications (six book chapters, 101 peer-reviewed scientific articles, and 11 peer-reviewed review articles) were retained for analysis.

3. Results

3.1. Earlier uses of animal discomfort and related concepts

The Merriam-Webster Dictionary (2023) defines discomfort as "mental or physical uneasiness" and the Cambridge Dictionary (2023) defines discomfort as "a feeling of being uncomfortable physically or mentally". Thesaurus synonyms found were "pain", "agony", "soreness", "ache", and "sting" (Cambridge Dictionary, 2023).

Of the literature retained for further analysis, only 25 publications explicitly embedded a concept or definition of animal discomfort or related concepts (e.g., pain, suffering, or sickness). The additional 93 publications dealt with measurement of discomfort in animals, including pigs, or measurement of related concepts specifically in pigs, without explicitly presenting any conceptualization or definition of animal discomfort or related concepts.

The concept-focused literature can be categorized within three different contexts: meat production ($N = 18$; 72 % of concept-focused literature), veterinary ($N = 5$; 20 % concept-focused literature), and biomedical ($N = 2$; 8 % concept-focused literature). Related to meat production, the animals explicitly involved were cattle (da Silva et al., 2022; Mainau et al., 2022; Moons et al., 2014; EFSA AHAW Panel, 2020; 2022b; 2023; Polsky and von Keyserlingk, 2017), goats (EFSA AHAW Panel, 2022f), sheep (EFSA AHAW Panel, 2022f), horses (EFSA AHAW Panel, 2022c), pigs (Forbes, 2009; Herskin et al., 2011; EFSA AHAW Panel, 2022d; 2022e), and aquatic species such as mollusks, crustaceans and fishes (Sneddon, 2015). Within the meat production field, four references did not specify the animal species when discussing or presenting a concept of discomfort or related term (Appleby, 2008; Broom, 1998; Mellor, 2016; EFSA AHAW Panel, 2022a). In the veterinary-related literature, the animal species explicitly cited were

rabbits (Banchi et al., 2020), pigs (Herskin and Di Giminiani, 2024), and harbor seals (Kastelein et al., 2006a). Within the veterinary field, two references did not specify the animal species when discussing or presenting a concept of discomfort or related term (Bee et al., 2020; Guesgen and Bench, 2017). In the biomedical-related literature, the animal species explicitly used were dogs (da Riz et al., 2021) and pigs (Brown and Gade, 2011). Two explicit definitions of discomfort were identified in the screened literature. The first definition was “discomfort can be physical or psychological and is characterized by an unpleasant feeling resulting in a natural response of avoidance or reduction of the source of the discomfort” (EFSA AHAW Panel, 2020; 2022a,2022b, 2022c,2022d,2022e;2022f; 2023), which was modified from a definition of human discomfort originally proposed by Ashkenazy and DeKeyser Ganz (2019) within a clinical nursing context. The second identified definition of discomfort was “minimal change in an animal’s adaptive level or comfort baseline as a result of changes in its environment or biological, physical, social, or psychological alterations”, which was presented as part of a discussion of stressful conditions in pig research (Brown and Gade, 2011).

Among the remaining concept-focused literature, no explicit definition of discomfort was identified. Instead, discomfort was used interchangeably with other negative affective states, such as pain (Banchi et al., 2020; Bee et al., 2020; Broom, 1998; Herskin and Giminiani, 2024; Herskin et al., 2011; Mainau et al., 2022; Mellor, 2016; Polsky and von Keyserlingk, 2017), suffering (Sneddon, 2015), malaise (Broom, 1998), or in terms of stress (Appleby, 2008; Mellor, 2016). Whilst, da Silva et al. (2022) presented a temperature-humidity index criterium to discuss the potential experience of thermal discomfort by cattle. Furthermore, da Riz et al. (2021) described discomfort as poor tolerance to esophageal stent placement for treatment of strictures in dogs. Meanwhile, Guesgen and Bench (2017) interpreted discomfort as absence of comfort and reported the interchangeable use of this term with a range of affective states such as frustration, boredom, fear, anxiety or a milder form of pain. Additionally, discomfort has been simply interpreted as a result of challenged homeostatic functions (Forbes, 2009; Kastelein et al., 2006a, b; Moons et al., 2014).

The literature review also revealed that animal discomfort and related concepts were used across three main domains, which could possibly overlap: physical/sensory discomfort, physiological discomfort, and mental discomfort (Table 1).

3.1.1. Physical/sensory featured discomfort

Several references used “animal discomfort” featured by a physical/

Table 1

Uses and domains of the term “animal discomfort” in the literature. The “X” indicates the domain(s) of each case. The cases appear in alphabetic order.

Use	Domain		
	Physical/Sensory	Physiological	Mental
Acoustic discomfort	X		
Anxiety			X
Body lesion	X		
Boredom			X
Fatigue	X		
Fear			X
Frustration			X
Health disorder	X		
Hunger	X	X	X
Inability to rest	X		
Inappetence		X	
Malaise		X	
Metabolic disorder		X	
Motion restraint	X		
Pain	X		
Suffering			X
Thermal discomfort		X	
Thirst	X	X	X

sensory component, with pain being the most frequently mentioned state either preceding or occurring simultaneously with discomfort (Banchi et al., 2020; da Riz et al., 2021; EFSA AHAW Panel, 2020; 2022a,2022b,2022c,2022d,2022e;2022f; 2023; Herskin and Di Giminiani, 2024; Herskin et al., 2011; Guesgen and Bench, 2017; Mainau et al., 2022; Mellor, 2016). According to the International Association for the Study of Pain, pain is defined as an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage or described in terms of it (Raja et al., 2020). As described in recent EFSA AHAW Panel scientific opinions (2020; 2022a,2022b,2022c,2022d,2022e,2022f; 2023), pain can be one of the causes of discomfort, but not every discomfort can be attributed to pain. In reality, other concepts often used interchangeably with discomfort in the physical/sensory domain were fatigue, thermal and acoustic impairment, body lesions, hunger, thirst, inability to rest, and mobility constrains (Forbes, 2009; da Silva et al., 2022; EFSA AHAW Panel, 2020; 2022a,2022b,2022c,2022d,2022e;2022f; 2023; Kastelein et al., 2006a,b; Polsky and von Keyserlingk, 2017). Specifically, in the body of literature explicitly referring to pigs, the typical sources of this type of discomfort were: overcrowding; unsuitable floors and pen features; visual, auditory or olfactory under/overstimulation; thwarted performance of comfort behaviors; exposure to extreme temperatures; damage to the integument or underlying tissues; infections; presence of ectoparasites or sunburn (Forbes, 2009; Brown and Gade, 2011; EFSA AHAW Panel, 2022d;2022e; Herskin and Di Giminiani, 2024; Herskin et al., 2011).

3.1.2. Physiologically featured discomfort

The concept of animal discomfort has also been presented or discussed when based on physiological features, often overlapping with physical/sensory features (Table 1). Animals typically manifest their discomfort through a combination of physiological and behavioral/physical mechanisms, with their response influenced by the nature of a given stressor as well as influenced by a complex interplay of genetic factors and past experiences (Brown and Gade, 2011). Physiologically featured discomfort in pigs, as well as in other domesticated animals, can, for instance, originate from: disturbed metabolism or infection; heat/cold stress; nutrient deficiency leading to hunger and thirst; presence of poisonous ectoparasites; inflammation of the navel, mammary gland(s) or any type of hernias; respiratory disorders; or eye disorders (Broom, 1998; da Silva et al., 2022; EFSA AHAW Panel, 2022d;2022e; Herskin et al., 2011; Polsky and von Keyserlingk, 2017). As part of the process of developing a framework for predicting feed intake in pigs, named Minimum Total Discomfort framework, Forbes (2009) discussed that, even though discomfort might be more obviously associated with physical causes such as stomach distension, an experience of discomfort can also be induced by metabolic factors such as (mild) excesses of metabolites or toxins. Forbes (2009) additionally posited that the physiological relevance of discomfort is independent of conscious experiencing and only a severe level of discomfort is brought to an animal’s attention. The latter contrasts with the aforementioned definition of discomfort proposed by Brown and Gade (2011). In reality, the distinction between physiologically featured discomfort and other forms of discomfort, such as mentally featured discomfort (see Section 3.1.3), is often nuanced and interrelated. For instance, individuals exposed to extreme heat may exhibit physiological responses, like sweating (in animal species that can sweat) and changes in body temperature regulation dynamics – responses that often are the ones that can be quantified in non-verbal animals –, indicating physiologically featured discomfort. However, if they are offered a possibility to seek shelter, they may do so even before the physiological responses are exacerbated based on their presumed memory of the consequences of being exposed to extreme heat. Hence, at a lower level, sources of physiologically featured discomfort may trigger behavioral responses first (Moons et al., 2014).

3.1.3. Mentally featured discomfort

Discomfort has also been presented as a mentally featured as an unpleasant negative affective state or a state of tension (Williams and Irurita, 2006), preceding or occurring with other more extensively-described negative affective states such as anxiety, boredom, fear, frustration or suffering (Sneddon, 2015; Mellor, 2016; Guesgen and Bench, 2017; Bee et al., 2020). Moons et al. (2014) suggested that a sense of discomfort can arise much sooner than clear physiological signs of distress arise. In this direction, distress can be theorized as a conscious, negatively valenced, intensified affective motivational state that occurs in response to a perception that current coping mechanisms (involving, partly, physiological stress responses) are at risk of failing to alleviate the aversiveness of the current situation in a sufficient and timely manner (McMillan, 2020). Intertwined with discomfort, suffering can be conceptualized as an affective state in which negative experiences dominate attention, there is limited capacity for distraction or compensation, normal life cannot be pursued, and full recovery cannot occur even if the external situation improves (Olsson et al., 2020). Aligned with this, Tate and Pearlman (2019) described the experience of suffering as adverse, profound, enduring, and transformative. In addition to the potential sources of physical/sensory and physiologically featured discomfort previously listed, in domesticated animals, mentally featured discomfort can originate from experimental routine procedures, such as blood sampling, social isolation, and exposure to novel environments and individuals during, among other contexts, transportation (e.g., Appleby, 2008; Brown and Gade, 2011; EFSA AHAW Panel, 2022d,2022e).

Among the many uses of animal discomfort found in literature, two uses were frequently overlapping among the three aforementioned domains: hunger and thirst (Table 1). Hunger and thirst can be defined as negative affective states caused by undernourishment and dehydration (more specifically for thirst) persisting longer than a usual inter-meal or visit to drinker interval (adapted from D'Eath et al., 2009). In the scientific literature it is not unusual to use hunger and thirst interchangeably with motivation to feed or drink (Kirkden and Pajor, 2006), so that these affective states are the indicated as drivers of feeding and drinking behavior. However, different from a motivation or short-term appetitive regulation, these negative affective states arise due to an individual's persistent inability to acquire nutrients and fulfill the energy or water demands. This continuous energy and/or water restriction can lead to physical (e.g., weight loss), physiological (e.g., ketosis), behavioral (e.g., increased frequency of vocalizations, signs increased feeding motivation), and/or cognitive changes (e.g., pessimistic perception of the environment, increased mental strain) (as reviewed by Kremer et al., 2020). In summary, hunger and thirst, while stemming from fundamental physiological states, leading to changes in motivational states, can have profound impacts on mental states.

3.2. Defining attributes

The term 'defining attributes' concerns the recurrently observed characteristics of a concept within the literature, exhibiting a consistent presence whenever the concept occurs (Walker and Avant, 2005). Based on this, the defining attributes of animal discomfort are: physical or mental negative affective state; unpleasant sensory or emotional experience; homeostatic mechanisms challenged by environmental changes and consequent biological, physical, or mental alterations; as well as behavioral signs of avoidance or attempt to reduce the source of discomfort.

3.3. Model case

According to Walker and Avant (2005), a model case involves the description of a real-life situation including all proposed attributes that exemplify the use of the concept. Accordingly, we constructed a model case for animal discomfort in pigs, which is described below:

Sixteen female pigs with an initial body weight of 50 kg were allocated in individual metabolic cages (Fig. 1) to study digestibility and the effect of two different diets on nitrogen and phosphorus excretion. The overall aim of the study was to find ways to mitigate environmental and climate impact of pig production. The cages were made of stainless steel with the dimensions of 0.5 × 1.5 m, with an elevated floor made of steel bars, a feed trough and drinking nipple. Feces dropped onto a metal plate placed below the floor of the cage. Pigs could move a few steps back and forth but were not able to turn around in the limited space. Pigs were fed twice daily and had ad libitum access to water. No straw or other enrichment material or toys were provided. After an adaptation period of 5 days, pigs were fitted with urine bladder catheters allowing separate collection of urine and feces for 5 days. Blood samples were collected on the last day (d 10) of the experiment by jugular vein puncture. During blood sampling, pigs were restrained with a nose snare placed around the upper jaw behind the canine teeth. During this procedure, most pigs were vocalizing with high pitch screams and trying to back away from the handler.

This is a typical case observed in pig nutrition experiments investigating, among others, digestibility and dietary effects on greenhouse gas emissions. Our model case meets the attributes inherent to animal discomfort: physical and/or mental aspects (limited space restricting movement and hindering exploration, impossibility to perform full behavioral repertoire and social isolation causing negative emotional stress); unpleasant sensory or emotional experience (arguably painful and stressful handling during blood sampling and placement of catheters); homeostatic mechanisms challenged by environmental changes (sudden and relatively long-lasting change of housing conditions); and behavioral signs of avoidance or attempt to reduce the source of discomfort (pigs vocalizing and backing away from the person restraining them).

3.4. Contrary case

Contrary cases are clear examples of what is not describing the concept (Walker and Avant, 2005). Hence, we constructed a contrary case related to pig production displaying fewer or no attributes of animal discomfort, which is stated below:

On an organic farm, pigs are raised in family pens (5–7 sows with their litters) on deep straw bedding with access to pasture. Pigs are fed ad libitum in a dry feeding system with several feeders in each pen and have constant access to water from water nipples. At 30 kg, litters of pigs are moved together to larger but similar deep bedded pens. Growing pigs are kept in the same family group until slaughter. Buildings are simple and uninsulated with natural ventilation, resulting in a very silent environment with good air quality but with indoor temperatures closely linked to outdoor conditions. In cold periods, pigs are often observed to lie close together and to bury themselves in the deep straw bedding. The farmer enters each pen every day and walks around calmly to check all pigs. Often, she throws out dried beans, wheat kernels or similar in the straw bedding to get resting pigs to raise voluntarily and to promote explorative behaviors. Pigs often gather around the farmer as soon as she enters the pen.

In contrast to the model case, pigs are here kept in stable groups at all times, fulfilling their needs of social contact and stability. With ad libitum feeding and water access, pigs are not likely to experience negative affective states such as hunger or thirst. Straw bedding and outdoor access provides good opportunities for expressing motivated species-specific behaviors such as rooting and exploration. The straw bedding also provides a suitable micro-climate for the pigs in spite of cold indoor temperatures during winter. Instead of avoiding human contact, pigs flock around the farmer as she enters the pen.

3.5. Identification of antecedents and consequences

According to Walker and Avant (2005), antecedents are the events or



Fig. 1. Illustration of cages used in pig nutrition and metabolism experimental studies at Aarhus University pig unit.

aspects that need to manifest before the occurrence of a concept; meanwhile, consequences are the events that can arise as a consequence of the occurrence of a concept and that can encourage novel research approaches to the concept in question. All physical (e.g., pain, body lesions, movement limitations), physiological (e.g., impaired metabolism, nutrient deficiency, thermal stress), and mental events (e.g., social isolation, fear, anxiety) eliciting mild-to-severe negative emotional experiences can be considered antecedents of discomfort. Whereas the consequences of discomfort are the animals' attempts to evade or minimize the source(s) of this affective state.

3.6. Identification of empirical referents

According to Walker and Avant (2005), empirical referents refer to the measurable indicators that represent the presence or absence of the concept in question and act as practical tools to quantify processes associated with the concept and their outcome. When analyzing the concept "discomfort" in animals, subjective empirical referents such as verbal communications cannot be used, thus the choice for objective empirical referents should be favored, but the qualitative behavior assessment (QBA), in which an observer subjectively assesses the manner an animal or a group of animals behave, has also been employed in animal affective state investigations (Wemelsfelder et al., 2000). It is important that indicators are non-invasive for the animals and feasible in scientific contexts.

Of the screened literature, we identified 93 publications in which discomfort or a related concept was quantified in animals (Table 2). The indicators to quantify discomfort used in these publications can be classified in three main categories: behavioral ($N = 75$; 57 % of the available literature), physical ($N = 34$; 26 % of the available literature), and physiological ($N = 23$; 17 % of the available literature). These three categories can overlap. Most of the publications only included one type

of indicator ($N = 61$, 66 % of the available literature), some included a combination of two types of indicators ($N = 25$; 27 % of the available literature), and a few included a combination of all three types of indicators ($N = 7$; 7 % of the available literature).

The behavioral category is related to a change of behavior of an animal in response to the presence of a stimulus. Among indicators are a wide range of species-specific behaviors (Irigoin-Lovera et al., 2019; Niittynen et al., 2022), activity and postural changes (Hall and Heleski 2017; Huisman et al., 2016; Swartz et al., 2023), avoidance or reactive behaviors (Górecka-Bruzda et al., 2015; Kreissl and Neiger 2015), changes in feeding behavior (Gigliuto et al., 2014; Gregorini et al., 2015), abnormal behaviors (EFSA AHAW Panel, 2022b,2022c,2022d, 2022e;2022f; Underwood et al., 2013), facial expressions (Swan et al., 2023; van Zeeland and Schoemaker 2023), vocalizations (Hillmann et al., 2004), or changes in comfort behavior (Desoubaux et al., 2018). The physical domain is related to pain and impaired health of the animals. Its indicators include decreased body weight (Duarte et al., 2021; Rix et al., 2020) and a wide variety of clinical signs such as muscle tremors (Hewawaduge et al., 2021; van Beirendonck et al., 2011), intense blinking (Acar et al., 2018; Pigatto et al., 2018), nasal/eye discharge (Pigatto et al., 2018), dirty fur (Rix et al., 2020), lameness (Richards et al., 2019), vomiting (Münster et al., 2013), changes in breathing (Liehr et al., 2017), fecal consistency (Littlewood and Mellor 2016), or pathology-specific signs (Rix et al., 2020). The physiological domain is related to changes in biological functions in response to stressors. Among indicators are indicators of productivity (Peana et al., 2017), electroencephalography (Gigliuto et al., 2014), cardiorespiratory variables (Hall and Heleski 2017; Imani et al., 2019), body temperature and blood pressure (Gigliuto et al., 2014), hematological variables such as plasma lactate or leukocyte count (Huisman et al., 2016), adrenal hormones such as cortisol and corticosterone (Herskin and Giminiani, 2024), and other hormones such as oxytocin (Niittynen et al., 2022).

Table 2

Retained body of literature focusing on empirical referents used to quantify discomfort in animals, including pigs, or related concepts specifically in pigs. For each reference the animal species of interest, context, treatment or situation that animals were subjected to, and empirical referents are presented.

Reference	Animal species	Context	What animals were subjected to	Empirical referent(s)
Acar et al., 2018	Rabbit	Biomedical	Liposomal suspension mimicking an artificial tear	Clinical status
Basile et al., 2015	Horse	Veterinary	Experimentally induced <i>Lyme borreliosis</i>	Activity
Beer et al., 2019	Cat	Veterinary	Ovariectomy procedure	Clinical status
Benato et al., 2021	Rabbit	Veterinary	Not specified	Activity
Bishop-Williams et al., 2015	Cattle	Veterinary	Heat stress	Clinical status
Black, 2009	Pig	Production	Not specified	Clinical status
Bruijn et al., 2012	Cattle	Production	Foot disorder	Clinical status
Caplen et al., 2012	Chicken	Veterinary	Lameness	Activity
Carstens and Moberg, 2000	Mouse / Rat / Guinea pig / Rabbit / Dog / Cat / Horse	Veterinary	Not specified	Activity, appearance, clinical status, respiratory parameters
Contreras-Aguilar et al., 2019	Pig	Veterinary	Lameness and prolapses	Activity, appearance, clinical status
d'Eath, 2012	Cattle	Veterinary	Lameness	Clinical status
Desoubeaux and Cray, 2018	Mouse / Rat / Guinea pig / Rabbit	Biomedical	Experimentally induced aspergillosis	Activity, appearance, body weight, body temperature
Desoubeaux et al., 2018	Rat	Biomedical	Experimentally induced aspergillosis	Activity, appearance, body weight
Dixon et al., 2016	Saker falcon	Veterinary	Harness-mounted satellite transmitters and patagial tags	Activity
Duarte et al., 2021	Mouse	Biomedical	Exposure to autologous tumor cells, bacillus Calmette-Guérin (BCG) and low concentrations of formalin	Activity, appearance, body weight
Fàbregas et al., 2021	Rhino	Veterinary	Herding	Activity, vocalization
Fentener van Vlissingen et al., 2015	Mouse	Biomedical	Not specified	Activity, appearance, body weight
Gigliuto et al., 2014	Cattle / Pig	Veterinary	Not specified	Activity, vocalization, cardiovascular parameters, respiratory parameters, body temperature, neurological parameters
Górecka-Bruzda et al., 2015	Horse	Production	Participation in elite equestrian competition	Activity
Götz and Janik, 2010	Seal	Veterinary	Playback of unpleasant sounds	Activity
Gregorini et al., 2015	Cattle	Production	Not specified	Activity
Hall and Heleski, 2017	Horse	Veterinary	Behavior of horse rider	Activity, hormones, body temperature, hematological parameters, cardiovascular parameters
Hausberger et al., 2016	Horse	Veterinary	Sickness	Activity
Hawkins et al., 2011	Rat	Veterinary	Not specified	Activity, cardiovascular parameters
Herskin and Giminiani, 2024	Pig	Veterinary	Not specified	Activity, vocalization, clinical status, hormones, body temperature, cardiovascular parameters, productivity
Hewawaduge et al., 2021	Mouse	Biomedical	Antacid formulations	Activity, clinical status
Hillmann et al., 2004	Pig	Production	Seasonal ambient temperatures	Vocalization
Huisman et al., 2016	Mouse	Biomedical	Irinotecan-induced toxicities	Activity, clinical status, appearance, hematological parameters
Häger et al., 2018	Mouse	Biomedical	Chemically induced acute colitis	Activity
Imani Rastabi et al., 2019	Dog	Veterinary	Epidural dexmedetomidine	Vocalization, cardiorespiratory parameters
Irgoin-Lovera et al., 2019	Guano bird	Veterinary	Presence of drones	Activity
Ison et al., 2016	Pig	Veterinary	Not specified	Activity, vocalization, hormones, neurological parameters
Kastelein et al., 2006a	Seal	Veterinary	Tone pulses generated by acoustic harassment devices	Activity
Kastelein et al., 2006b	Seal / Trout	Veterinary	Sounds generated by acoustic data communication network	Activity
Khol et al., 2019	Cattle	Veterinary	Sub-clinical ketosis	Activity
Kreissl and Neiger, 2015	Dog	Veterinary	Low, normal, or high body temperatures	Activity
Ladewig et al., 2022	Horse	Veterinary	Not specified	Activity
Lanci et al., 2022	Horse	Veterinary	Not specified	Facial expression
Landa, 2012	Pig	Veterinary	Not specified	Activity, clinical status, body weight, hormones, cardiovascular parameters, respiratory parameters
Lechner et al., 2021	Pig	Production	CO ₂ stunning	Activity
Levionnois et al., 2018	Horse	Veterinary	Colon constipation	Clinical status
Li et al., 2015	Medaka ricefish	Veterinary	Varying ambient temperatures	Productivity, body temperature
Liao et al., 2014	Rat	Biomedical	Experimental tooth movement	Facial expression
Liehr et al., 2017	Pig	Veterinary	Experimentally-induced chronic gut inflammation	Activity, body weight, clinical status, body temperature
Littlewood and Mellor, 2016	Dog	Veterinary	Not specified	Activity, clinical status
Luna et al., 2020	Pig	Production	Orchiectomy	Activity
Maia et al., 2013	Horse	Veterinary	Thermal stress	Body temperature, cardiovascular parameters, respiratory parameters
Mainau and Manteca, 2011	Cattle / Pig	Production	Parturition	Activity, vocalizations, body weight, body temperature, hormones, cardiovascular parameters
Maravilla et al., 2011	Chinchilla	Biomedical	<i>Taenia solium</i> taeniasis	Activity, body weight, clinical status
Martin et al., 2019	Horse	Biomedical	Oral misoprostol	Activity

(continued on next page)

Table 2 (continued)

Reference	Animal species	Context	What animals were subjected to	Empirical referent(s)
Mbuthia et al., 2021	Cattle	Production	Heat stress	Clinical status
Menchetti et al., 2021	Sheep	Production	Reduced space allowance and heat stress	Activity, eye temperature
Millicamps et al., 2012	Mouse	Biomedical	Invertebral disc degeneration	Activity
Moons et al., 2015	Cattle	Production	Heat stress	Activity, respiratory parameters
Münster et al., 2013	Dog	Veterinary	Esophageal disease	Activity, clinical status
Nicetto and Longo, 2019	Dog	Veterinary	Traumatic appendicular bone injuries	Clinical status
Niittynen et al., 2022	Horse	Veterinary	Foundation training sessions	Activity, hormones
Ouahrani-Bettache et al., 2019	Mouse	Biomedical	<i>E. coli</i> and <i>Brucella</i> strains	Activity, appearance, body weight, clinical status
Panteleeva et al., 2013	Mouse	Veterinary	Experimental risky hunting situation	Activity
Peana et al., 2017	Sheep	Production	Winter and spring meteorological conditions	Mily yield
Petit et al., 2017	Slugs	Biomedical	Exposure to canine and human shampoos	Clinical status
Piccolo et al., 2019	Cat	Veterinary	<i>Toxoplasma gondii</i> infection	Clinical status
Pigatto et al., 2018	Horse	Veterinary	Conjunctival melanoma	Clinical status
Pilz et al., 2012	Cattle	Veterinary	Vaginal examination	Activity, vocalization
Pinna et al., 2015	Emperor tamarin	Veterinary	Yogurt dietary supplementation	Clinical status
Plesch et al., 2010	Cattle	Production	Resting and disturbance periods	Activity
Post et al., 2012	Pig	Biomedical	Not specified	Clinical status
Price and Nolan, 2001	Sheep	Veterinary	Castration and tail docking	Activity
Rault et al., 2011	Pig	Production	Castration	Activity, hormones, cardiovascular parameters
Richards et al., 2019	Rat	Biomedical	Patch clamp mimicking dynamics of gepapixant	Clinical status
Rix et al., 2020	Mouse	Veterinary	Chemotherapy	Activity, body weight, clinical status
Schlicht and Kempenaers, 2018	Blue tit	Veterinary	Ringling, blood sampling and PIT-tag implanting	Activity, vocalization
Schwartzkopf-Genswein et al., 1997	Chicken	Veterinary	Hot-iron and freeze branding	Activity, clinical status, hormones
Sneddon et al., 2003	Trout	Veterinary	Not specified	Cardiovascular parameters
Stefanowska et al., 2000	Cattle	Production	Milking omission	Activity
Swan et al., 2023	Mouse	Biomedical	Repeated experimental handling	Facial expression
Swartz et al., 2023	Cattle	Production	Dexamethasone administration	Activity
Tétreault et al., 2011	Rat	Biomedical	von Frey and Dynamic Weight Bearing device	Activity
Underwood et al., 2013	Cattle	Production	Not specified	Activity, vocalization, hormones
Upchurch et al., 2016	Dog	Veterinary	Administration of adipose-derived stromal vascular fraction and platelet-rich plasma	Clinical status
Van Beirendonck et al., 2011	Pigs	Production	Bundling and/or anesthesia	Activity, clinical status
van Zeeland and Schoemaker, 2023	Ferret	Veterinary	Not specified	Facial expression
Vicario-de-la-Torre et al., 2018	Rabbit	Biomedical	Dry eye	Vocalization, clinical status
von Borstel et al., 2009	Horse	Production	Riding-occurred Rollkur (i.e., hyperflexion of the horse's neck)	Activity, cardiovascular parameters
Wang et al., 2021	Pig	Biomedical	Wired or wireless Fecobionics devices	Activity
Whittaker and Howarth, 2014	Mouse	Veterinary	Not specified	Activity
Zhang et al., 2017	Pig	Production	Confinement	Activity, hormones

These indicators have been used to quantify discomfort in a wide variety of species. In pig research, behavioral indicators are most commonly used. This work has established that a higher number of posture changes (EFSA AHAW 2022d; Rault et al., 2011; van Beirendonck et al., 2011), escape attempts (Lechner et al., 2021), and a decreased feed and/or water intake (Rault et al., 2011) are indicative of discomfort, as well as vocalizations such as high-frequency calls (Hillmann et al., 2004) or other calls induced by stressors. Regarding the physical indicators, decreased body weight (Liehr et al., 2017), the presence of lesions or lameness (Herskin and Giminiani, 2024), spasms or shivering (van Beirendonck et al., 2011), abnormal feces or breathing pattern, nasal discharge (Liehr et al., 2017), and latency time of pupil constriction (Zhang et al., 2017) are used as measurable indicators of discomfort in pigs. The physiological indicators for quantification of discomfort in pigs includes increased levels of cortisol and/or adrenocorticotropic hormone (Gigliuto et al., 2014; Zhang et al., 2017, Contreras-Aguilar 2009), variations in body temperature and blood pressure (Gigliuto et al., 2014), impaired immune function (Rault et al., 2011), increased heart rate and respiratory rate as well as abnormal electroencephalogram-activity (Gigliuto et al., 2014).

In addition to separate measures of different variables, aggregated scoring systems for assessing pain, distress, and discomfort in pigs have been developed (Contreras-Aguilar et al., 2019; Liehr et al., 2017) based on the system that Morton and Griffiths (1985) proposed for laboratory

rodents. For example, Contreras-Aguilar et al. (2019) proposed a system that combines different indicators and consists of 5 independent variable categories: unprovoked behavior, behavioral responses to external stimuli, appearance, body condition score, and clinical signs. According to the system, these variables can be scored from 0 to 3 obtaining a maximum score of 20 and classifying the animals as not showing (total score of 0–4) or showing signs of distress and discomfort (total score of 5–20). This type of scoring systems adapted to each intervention, together with additional behavioral, physical, or physiological indicators was advised by the authors as a way to quantify discomfort in pigs.

3.7. Proposed operational definition

Based on our literature review, model case, and identified antecedents, consequences and empirical referents, we propose the following operational definition of animal discomfort: short- or long-lived negative affective state featured by physical, physiological and/or mental components, induced by internal or external stimuli, ranging from mild to severe, potentially occurring together with other negative affective states, and leading to avoidance or attempt to alleviate the source of uneasiness.

4. Discussion

The Walker and Avant (2005) concept analysis approach is used to refine unclear terms and provide operational definitions of a specific term with a clear theoretical basis. We followed this approach rigorously by including scientific materials pertaining to the fields of biomedicine, veterinary science, animal science, and biology. Each document was screened based on our pre-determined inclusion criteria. The latter is particularly important to reduce the risk of overlooking relevant materials and likely places our review in advantage compared to earlier studies applying the Walker and Avant (2005) concept analysis methodology, as initial screening of titles and abstracts of selected materials may result in relevant information potentially being missed. In literature, there are examples of reports not disclosing the literature screening approach or the actual number of publications retained after literature analysis (e.g., psychological distress – Ridner, 2004; overcoming – Brush et al., 2011). Despite the thorough literature search and screening, we only found 25 animal-focused publications explicitly containing a concept or definition of animal discomfort, which is a considerably low number compared to the growing body of literature claiming to be investigating this negative affective state and given the increasing societal request for more ethical, welfare-oriented management of animals kept for production or biomedical purposes. Thus, this review not only serves as a guide for detection, interpretation, and assessment of animal discomfort, but also sheds light on the need for more scientific attention to the occurrence of this state.

Our operational definition holds valuable scientific, ethical, and practical significance and can assist in establishing clearer standards and guidelines to safeguard the welfare of domesticated animals kept for scientific purposes as well as for livestock production. Discomfort encompasses a range of physical, physiological and mental components. Identifying and characterizing these components can help recognize subtle physiological and/or behavioral changes that animals can potentially display, enabling early and proper intervention. Furthermore, scientifically defining the term ‘animal discomfort’ can facilitate the development of animal-welfare-oriented protocols and regulations, accurate assessment of ethical permit applications for animal experimentation, and improve transparency of animal experimentation and livestock farming (Kiani et al., 2002; Mello, 2012; Tannenbaum and Bennett, 2015; PIGWEB, 2024). Aggregated scoring systems for assessing discomfort in pigs within research are already available (Liehr et al., 2017; Contreras-Aguilar et al., 2019) and could be developed further.

The implications of defining, understanding, and measuring discomfort in domesticated animals extend beyond ethical considerations; and are intrinsic to a successful green transition to sustainable livestock production (at all stages – on-farm, during transport and slaughter) and agriculture (Olesen et al., 2021). By prioritizing animal welfare through a comprehensive understanding and assessment, we can potentially identify and correct discomfort-inducing aspects to alleviate animal discomfort within farm management practices and research methods, aiming to make animal experimentation and animal-derived food production more ethical, efficient, and with less environmental impact. In this regard, future precision livestock farming tools can potentially detect sources of discomfort at early stages of occurrence, facilitating quick intervention and prevention of further adverse and more serious welfare conditions (Neethirajan, 2022).

5. Conclusion

We used the Walker and Avant (2005) concept analysis method to conceptualize the term “animal discomfort”. We propose that animal discomfort can be conceptualized as a short- or long-lived negative affective state featured by physical, physiological and/or mental components, induced by internal or external stimuli, ranging from mild to severe, potentially occurring together with other negative affective states, and leading to avoidance or attempts to lessen the source of

uneasiness. This suggestion has the potential to serve as the foundation for establishing a more standardized application and comprehension of the concept, potentially facilitating the inference into the negative affective states experienced by pigs as well as other domesticated animals kept for commercial farming and scientific purposes. Consequently, this proposed definition and the implementation of the already existing aggregated systems to quantify discomfort or further developed ones, have the potential to bolster legislative consistency and research integrity across various contexts, including animal model studies, biomedical and veterinary clinical investigations, feed efficiency and digestibility trials as well as ethological studies.

CRedit authorship contribution statement

Guilherme A. Franchi: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Marc Bagaria:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Heleen Boswijk:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Emma Fàbrega:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization. **Mette S. Herskin:** . **Rebecka Westin:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

This manuscript was developed as part of project PIGWEB funded by the European Union’s Horizon 2020 research and innovation program under grant agreement No. 101004770. All authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This manuscript was developed as part of project PIGWEB funded by the European Union’s Horizon 2020 research and innovation program under grant agreement No. 101004770.

References

- Acar, D., Molina-Martínez, I.T., Gómez-Ballesteros, M., Guzmán-Navarro, M., Benítez-del-Castillo, J.M., Herrero-Vanrell, R., 2018. Novel liposome-based and in situ gelling artificial tear formulation for dry eye disease treatment. *Contact Lens Anterior. Eye* 41, 93–96.
- Almond, G.W., 1996. Research applications using pigs. *the veterinary clinics of North America. Food Anim. Pract.* 12, 707–716.
- Science of animal welfare. In: Appleby, M.C., Appleby, M.C., et al. (Eds.), 2008. *Long Distance Transport and Welfare of Farm Animals*. Taylor & Francis Group, pp. 1–17.
- Ashkenazy, S., Ganz, F.D., 2019. The differentiation between pain and discomfort: a concept analysis of discomfort. *Pain Manag. Nurs.* 20, 556–562.
- Augère-Granier, M.-L., 2020. Briefing: the EU pig meat sector. *European Parliamentary Res. Service*. [https://www.europarl.europa.eu/RegData/etudes/BRIE/2020/652044/EPRS_BRI\(2020\)652044_EN.pdf](https://www.europarl.europa.eu/RegData/etudes/BRIE/2020/652044/EPRS_BRI(2020)652044_EN.pdf). Accessed on Sep 21, 2023.
- Banchi, P., Quaranta, G., Ricci, A., Mauthe von Degerfeld, M., 2020. Reliability and construct validity of a composite pain scale for rabbit (CANCERS) in a clinical environment. *PLoS ONE* 15 (4), e0221377.
- Basile, R.C., Rivera, G.G., Del Rio, L.A., de Bonis, T.C.M., do Amaral, G.P.D., Giangreco, E., Ferraz, G., Yoshinari, N.H., Canola, P.A., Queiroz Neto, A., 2015. Anaphylactoid reaction caused by sodium ceftriaxone in two horses experimentally infected by *Borrelia burgdorferi*. *BMC Vet. Res.* 11, 1–9.
- Bee, M., Bernal, X., Calisi, R., Carere, C., Carter, T., Fuertbauer, L., Smith, I.I.A., Sneddon, L., Vitale, A., 2020. Guidelines for the treatment of animals in behavioural research and teaching. *Anim. Behav.* 159.
- Beer, A.J.C., Lipscomb, V.J., Rutherford, L., Lee, K.C.L., 2019. Use of subcutaneous ureteral bypass systems as a bridge to definitive ureteral repair in a cat with bilateral ureteral ligation secondary to complicated ovariohysterectomy. *Vet. Rec. Case Rep.* 7, e000758.

- Benato, L., Murrell, J., Knowles, T.G., Rooney, N.J., 2021. Development of the Bristol Rabbit Pain Scale (BRPS): a multidimensional composite pain scale specific to rabbits (*Oryctolagus cuniculus*). *PLoS ONE* 16, e0252417.
- Bishop-Williams, K.E., Berke, O., Pearl, D.L., Hand, K., Kelton, D.F., 2015. Heat stress related dairy cow mortality during heat waves and control periods in rural Southern Ontario from 2010 to 2012. *BMC Vet. Res.* 11, 1–10.
- Black, J.L., 2009. Models to predict feed intake. *Voluntary Feed Intake in Pigs*. Wageningen Academic, pp. 323–351.
- Bruijnjs, M.R.N., Beerda, B., Hogeveen, H., Stassen, E.N., 2012. Assessing the welfare impact of foot disorders in dairy cattle by a modeling approach. *Animal* 6, 962–970.
- Brush, B.L., Kirk, K., Gultekin, L., Baiardi, J.M., 2011. Overcoming: a Concept Analysis. *Nurs. Forum* 46, 160–168.
- Broom, D.M., 1998. Welfare, stress, and the evolution of feelings. *Advances in the Study of Behavior: Stress and Behavior*. Academic Press, pp. 371–402.
- Brown, L.D., Gade, L.P., et al., 2011. Stress in experiments: stress issues in porcine research. In: McNulty, P.A., et al. (Eds.), *The Minipig in Biomedical Research*. Taylor & Francis Group, pp. 94–116.
- Cambridge Dictionary. (n.d.). Discomfort. in [cambridge-dictionary.com dictionary](https://dictionary.cambridge.org/dictionary/english/discomfort). <https://dictionary.cambridge.org/dictionary/english/discomfort> (Retrieved October 9, 2023).
- Caplen, G., Hotherhall, B., Murrell, J.C., Nicol, C.J., Waterman-Pearson, A.E., Weeks, C.A., Colborne, G.R., 2012. Kinematic analysis quantifies gait abnormalities associated with lameness in broiler chickens and identifies evolutionary gait differences. *PLoS ONE* 7, e40800.
- Carstens, E., Moberg, G.P., 2000. Recognizing pain and distress in laboratory animals. *ILAR J.* 41, 62–71.
- Contreras-Aguilar, M.D., Escibano, D., Martínez-Miró, S., López-Arjona, M., Rubio, C.P., Martínez-Subiela, S., Cerón, J.J., Tecles, F., 2019. Application of a score for evaluation of pain, distress and discomfort in pigs with lameness and prolapses: correlation with saliva biomarkers and severity of the disease. *Res. Vet. Sci.* 126, 155–163.
- Da Riz, F., Béguin, J., Manassero, M., Faucher, M., Freiche, V., 2021. Outcome of dogs and cats with benign oesophageal strictures after balloon dilatation or stenting: 27 cases (2002–2019). *J. Small Anim. Pract.* 62, 886–894.
- da Silva, V.C., de Sousa Nascimento, R., Neto, J.P.L., de Melo Lopes, F.F., Miranda, J.R., Furtado, D.A., 2022. Animal thermal comfort index for the state of Paraíba, Brazil: trend, influencing factors, and mitigating measures. *Theor. Appl. Climatol.* 147, 523–534.
- Dawkins, M.S., 1988. Behavioural deprivation: a central problem in animal welfare. *Appl. Anim. Behav. Sci.* 20, 209–225.
- d'Eath, R.B., 2012. Repeated locomotion scoring of a sow herd to measure lameness: consistency over time, the effect of sow characteristics and inter-observer reliability. *Anim. Welf.* 21, 219–231.
- D'Eath, R.B., Tolkamp, B.J., Kyriazakis, I., Lawrence, A.B., 2009. 'Freedom from hunger' and preventing obesity: the animal welfare implications of reducing food quantity or quality. *Anim. Behav.* 77, 275–288.
- Desoubeaux, G., Chauvin, D., Del Carmen Piqueras, M., Bronson, E., Bhattacharya, S.K., Sirpensi, G., Bailly, E., Cray, C., 2018. Translational proteomic study to address host protein changes during aspergillosis. *PLoS ONE* 13, e0200843.
- Desoubeaux, G., Cray, C., 2018. Animal models of aspergillosis. *Comp. Med.* 68, 109–123.
- Dixon, A., Ragyov, D., Purev-Ochir, G., Rahman, M.L., Batbayar, N., Bruford, M.W., Zhan, X., 2016. Evidence for deleterious effects of harness-mounted satellite transmitters on saker falcons *falco cherrug*. *Bird Study* 63, 96–106.
- Douglas, W.R., 1972. Of pigs and men and research: a review of applications and analogies of the pig, *sus scrofa*, in human medical research. *Space Life Sci.* 3, 226–234.
- Duarte, C., M. A. Carballo, O., J. M. De Gouveia, Y.M., García, A., Ruiz, Convit, A.F., 2021. Toxicity evaluation of ConvitVax breast cancer immunotherapy. *Sci. Rep.* 11, 12669.
- EFSA Panel on Animal Health and Welfare (AHAW), Nielsen, S.S., Alvarez, J., Bicout, D. J., Calistri, P., Canali, E., Winckler, C., 2022a. Methodological guidance for the development of animal welfare mandates in the context of the farm to fork strategy. *EFSA J.* 20, e07403.
- EFSA Panel on Animal Health and Welfare (AHAW), Nielsen, S.S., Alvarez, J., Bicout, D. J., Calistri, P., Depner, K., Winckler, C., 2020. Welfare of cattle at slaughter. *EFSA J.* 18, e06275.
- EFSA Panel on Animal Health and Welfare (AHAW), Nielsen, S.S., Alvarez, J., Bicout, D. J., Calistri, P., Canali, E., Herskin, M., 2022b. Welfare of cattle during transport. *EFSA J.* 20, e07442.
- EFSA Panel on Animal Health and Welfare (AHAW), Nielsen, S.S., Alvarez, J., Bicout, D. J., Calistri, P., Canali, E., Herskin, M., 2022c. Welfare of equidae during transport. *EFSA J.* 20, e07444.
- EFSA Panel on Animal Health and Welfare (AHAW), Nielsen, S.S., Alvarez, J., Bicout, D. J., Calistri, P., Canali, E., Spoolder, H., 2022d. Welfare of pigs on farm. *EFSA J.* 20, e07421.
- EFSA Panel on Animal Health and Welfare (AHAW), Nielsen, S.S., Alvarez, J., Bicout, D. J., Calistri, P., Canali, E., Herskin, M., 2022e. Welfare of pigs during transport. *EFSA J.* 20, e07445.
- EFSA Panel on Animal Health and Welfare (AHAW), Nielsen, S.S., Alvarez, J., Bicout, D. J., Calistri, P., Canali, E., Herskin, M., 2022f. Welfare of small ruminants during transport. *EFSA J.* 20, e07404.
- EFSA Panel on Animal Health and Animal Welfare (AHAW), Nielsen, S.S., Alvarez, J., Bicout, D.J., Calistri, P., Canali, E., Winckler, C., 2023. Welfare of calves. *EFSA J.* 21, e07896.
- Eskildsen, M., Hedemann, M.S., Theil, P.K., Nørgaard, J.V., 2020. Impact of substituting compound feed with increasing levels of fresh grass-clover on nitrogen metabolism and plasma metabolites of sows. *Livest. Sci.* 242, 104269.
- European Animal Research Association (EARA). (2023). *Pigs and biomedical research*. <https://www.eara.eu/pigs-and-animal-research> (Accessed on Sep 21, 2023).
- Fàbregas, M.C., Fosgate, G.T., Ganswindt, A., Bertschinger, H., Meyer, L.C., 2021. Unforeseen consequences of conservation management practices: case study on herding rhino as an anti-poaching measure. *Anim. Conserv.* 24, 412–423.
- Fentener van Vlissingen, J.M., Borrens, M., Girod, A., Lelovas, P., Morrison, F., Torres, Y. S., 2015. The reporting of clinical signs in laboratory animals: FELASA Working Group Report. *Lab. Anim.* 49, 267–283.
- Forbes, J.M., 2009. Integration of pre- and post-absorptive factors in feed intake regulation and prediction with particular respect to the pig. In: Torralardona, David, Roura, Eugeni (Eds.), *Voluntary Feed Intake in Pigs*. Wageningen Academic Publishers, pp. 61–86.
- Friend, D.W., MacIntyre, T.M., 1969. Metabolism cage design for male and female pigs. *Can. J. Anim. Sci.* 49, 130–131.
- Gigliuto, C., De Gregori, M., Malafoglia, V., Raffaelli, W., Compagnone, C., Visai, L., Petri, P., Avanzini, M.A., Muscoli, C., Viganò, J., Calabrese, F., Dominioni, T., Allegri, M., Cobiainchi, L., 2014. Pain assessment in animal models: do we need further studies? *J. Pain. Res.* 7, 227–236.
- Górecka-Bruzda, A., Kosińska, I., Jaworski, Z., Jeziński, T., Murphy, J., 2015. Conflict behavior in elite show jumping and dressage horses. *J. Vet. Behav.* 10, 137–146.
- Götz, T., Janik, V.M., 2010. Aversiveness of sounds in phocid seals: psycho-physiological factors, learning processes and motivation. *J. Exp. Biol.* 213, 1536–1548.
- Gregorini, P., Villalba, J.J., Provenza, F.D., Beukes, P.C., Forbes, J.M., 2015. Modelling preference and diet selection patterns by grazing ruminants: a development in a mechanistic model of a grazing dairy cow, MINDY. *Anim. Prod. Sci.* 55, 360.
- Guesgen, M.J., Bench, C.J., 2017. What can kinematics tell us about the affective states of animals? *Anim. Welf.* 26, 383–397.
- Hall, C., Heleski, C., 2017. The role of the ethogram in equitation science. *Appl. Anim. Behav. Sci.* 190, 102–110.
- Hausberger, M., Fureix, C., Lesimple, C., 2016. Detecting horses' sickness: in search of visible signs. *Appl. Anim. Behav. Sci.* 175, 41–49.
- Hawkins, P., Morton, D.B., Burman, O., Dennison, N., Honess, P., Jennings, M., Westwood, K., 2011. A guide to defining and implementing protocols for the welfare assessment of laboratory animals: eleventh report of the BVAAWF/FRAME/RSPCA/UFUW Joint working group on refinement. *Lab. Anim.* 45, 1–13.
- Herskin, M.S., Bonde, M.K., Jørgensen, E., Jensen, K.H., 2011. Decubital shoulder ulcers in sows: a review of classification, pain and welfare consequences. *Animal* 5, 757–766.
- Herskin, M.S., Bundgaard, C.J., Ottesen, J.L., Sørensen, D.B., Marchant-Forde, J.N., 2020. The pig. *Animal-centric Care and Management* 173–186.
- Herskin, M.S., Di Giminiani, P., 2024. Advances in pig welfare. In: Camerlink, I., Baxter, E.M. (Eds.), *Pain in pigs: Characterisation and Indicators*. Woodhead Publishing, pp. 23–48.
- Herskin, M.S., Franchi, G.A., Jensen, M.B., 2018. Animal discomfort—Are you comfortable with this term?. In: PSN027, poster presented at the World Congress of the IASP, September 12–16. Boston, MA.
- Hewawaduge, C., Seneviratne, A., Yang, M.-S., Jeong, T.-W., Kim, B., Lee, J.H., 2021. Comparative study of sodium bicarbonate- and magnesium hydroxide-based gastric antacids for the effectiveness of Salmonella delivered Brucella antigens against wild type challenge in BALB/c mice. *Pathog. Dis.* 79, ftab002.
- Hillmann, E., Mayer, C., Schön, P.C., Puppe, B., Schrader, L., 2004. Vocalisation of domestic pigs (*Sus scrofa domestica*) as an indicator for their adaptation towards ambient temperatures. *Appl. Anim. Behav. Sci.* 889, 195–206.
- Huisman, S.A., De Bruijn, P., Ghobadi Moghaddam-Helmantel, I.M., Ijzermans, J.N.M., Wiemer, E.A.C., Mathijssen, R.H.J., De Bruin, R.W.F., 2016. Fasting protects against the side effects of irinotecan treatment but does not affect anti-tumour activity in mice. *Br. J. Pharmacol.* 173, 804–814.
- Häger, C., Keubler, L.M., Talbot, S.R., Biernot, S., Weegh, N., Buchheister, S., Bleich, A., 2018. Running in the wheel: defining individual severity levels in mice. *PLoS Biol.* 16, e2006159.
- Imani Rastabi, H., Naddaf, H., Mosallanejad, B., Khajeh, A., 2019. Evaluation of the feasibility, reversibility and cardiorespiratory effects of epidural dexmedetomidine in sedated dogs undergoing orchiectomy. *Kafkas Univ. Vet. Fak. Derg.* 25, 807–813.
- Irigoin-Lovera, C., Luna, D.M., Acosta, D.A., Zavalaga, C.B., 2019. Response of colonial Peruvian guano birds to flying UAVs: effects and feasibility for implementing new population monitoring methods. *PeerJ* 7, e8129 do.
- Ison, S.H., Clutton, R.E., Di Giminiani, P., Rutherford, K.M., 2016. A review of pain assessment in pigs. *Front. Vet. Sci.* 3, 108.
- Kastelein, R.A., van der Heul, S., Terhune, J.M., Verboom, W.C., Triesscheijn, R.J., 2006a. Deterring effects of 8–45 kHz tone pulses on harbour seals (*Phoca vitulina*) in a large pool. *Mar. Environ. Res.* 62, 356–373.
- Kastelein, R.A., van der Heul, S., Verboom, W.C., Triesscheijn, R.J., Jennings, N.V., 2006b. The influence of underwater data transmission sounds on the displacement behaviour of captive harbour seals (*Phoca vitulina*). *Mar. Environ. Res.* 61, 19–39.
- Khol, J.L., Freigassner, K., Stanitznig, A., Tichy, A., Wittek, T., 2019. Evaluation of a handheld device for the measurement of beta-hydroxybutyrate in capillary blood obtained by the puncture of the vulva as well as in venous whole blood in cattle. *Pol. J. Vet. Sci.* 557–564.
- Kiani, A.K., Pheby, D., Henahan, G., Brown, R., Sieving, P., Sykora, P., Marks, R., Falsini, B., Capodicasa, N., Miertus, S., Lorusso, L., Dondossola, D., Tartaglia, G.M., Ergoren, M.C., Dundar, M., Michelini, S., Malacarne, D., Bonetti, G., Dautaj, A., Donato, K., International Bioethics Study Group, 2022. Ethical considerations regarding animal experimentation. *J. Prev. Med. Hyg.* 63, 255–266.

- Kirkden, R.D., Pajor, E.A., 2006. Using preference, motivation and aversion tests to ask scientific questions about animals' feelings. *Appl. Anim. Behav. Sci.* 100, 29–47.
- Kreissl, H., Neiger, R., 2015. Measurement of body temperature in 300 dogs with a novel noncontact infrared thermometer on the cornea in comparison to a standard rectal digital thermometer. *J. Vet. Emerg. Crit. Care* 25, 372–378.
- Kremer, L., Holkenborg, S.K., Reimert, I., Bolhuis, J.E., Webb, L.E., 2020. The nuts and bolts of animal emotion. *Neurosci. Biobehav. Rev.* 113, 273–286.
- Ladewig, J., McLean, A.N., Wilkins, C.L., Fenner, K., Christensen, J.W., McGreevy, P.D., 2022. A review of the ridden horse pain ethogram and its potential to improve ridden horse welfare. *J. Vet. Behav.* 54, 54–61.
- Lanci, A., Benedetti, B., Freccero, F., Castagnetti, C., Mariella, J., van Loon, J.P., Padalino, B., 2022. Development of a composite pain scale in foals: a pilot study. *Animals* 12, 439.
- Landa, L., 2012. Pain in domestic animals and how to assess it: a review. *Vet. Med. (Praha)* 57.
- Lechner, I., Léger, A., Zimmermann, A., Atkinson, S., Schuppers, M., 2021. Discomfort period of fattening pigs and sows stunned with CO₂: duration and potential influencing factors in a commercial setting. *Meat Sci.* 179, 108535.
- Levionnois, O.L., Graubner, C., Spadavecchia, C., 2018. Colon constipation in horses after sustained-release buprenorphine administration. *Vet. Anaesth. Analg.* 45, 876–880.
- Li, A.J., Leung, P.T., Bao, V.W., Lui, G.C., Leung, K.M., 2015. Temperature-dependent physiological and biochemical responses of the marine medaka *Oryzias melastigma* with consideration of both low and high thermal extremes. *J. Therm. Biol.* 54, 98–105.
- Liao, L., Long, H., Zhang, L., Chen, H., Zhou, Y., Ye, N., Lai, W., 2014. Evaluation of pain in rats through facial expression following experimental tooth movement. *Eur. J. Oral Sci.* 122, 121–124.
- Liehr, M., Mereu, A., Pastor, J.J., Quintela, J.C., Staats, S., Rimbach, G., Ipharraguerre, I. R., 2017. Olive oil bioactives protect pigs against experimentally-induced chronic inflammation independently of alterations in gut microbiota. *PLoS ONE* 12, e0174239.
- Littlewood, K.E., Mellor, D.J., 2016. Changes in the welfare of an injured working farm dog assessed using the five domains model. *Animals* 6, 58.
- Luna, S.P.L., de Araújo, A.L., da Nóbrega Neto, P.I., Brondani, J.T., de Oliveira, F.A., Azerêdo, L.M.D.S., Trindade, P.H.E., 2020. Validation of the UNESP-Botucatu pig composite acute pain scale (UPAPS). *PLoS ONE* 15, e0233552.
- Maia, A.P.D.A., Oliveira, S.R.D.M., Moura, D.J.D., Sarubbi, J., Vercellino, R.D.A., Medeiros, B.B.L., Griska, P.R., 2013. A decision-tree-based model for evaluating the thermal comfort of horses. *Sci. Agric.* 70, 377–383.
- Mainau, E., Llonch, P., Temple, D., Goby, L., Manteca, X., 2022. Alteration in activity patterns of cows as a result of pain due to health conditions. *Animals* 12, 176.
- Mainau, E., Manteca, X., 2011. Pain and discomfort caused by parturition in cows and sows. *Appl. Anim. Behav. Sci.* 135, 241–251.
- Maravilla, P., Garza-Rodriguez, A., Gomez-Diaz, B., Jimenez-Gonzalez, D.E., Toral-Bastida, E., Martinez-Ocaña, J., Flisser, A., 2011. Chinchilla laniger can be used as an experimental model for *Taenia solium* taeniasis. *Parasitol. Int.* 60, 364–370.
- Martin, E.M., Schirmer, J.M., Jones, S.L., Davis, J.L., 2019. Pharmacokinetics and ex vivo anti-inflammatory effects of oral misoprostol in horses. *Equine Vet. J.* 51 (3), 415–421.
- Mbuthia, J.M., Mayer, M., Reinsch, N., 2021. Modeling heat stress effects on dairy cattle milk production in a tropical environment using test-day records and random regression models. *Animal* 15, 100222.
- McMillan, F., 2020. Mental health and well-being in animals. In: McMillan, F. (Ed.), *What is distress? A complex Answer to a Simple Question*, 2nd Edition. CAB International, pp. 140–155.
- Mellor, D.J., 2012. Animal emotions, behaviour and the promotion of positive welfare states. *N Z Vet J* 60, 1–8.
- Mellor, D.J., 2016. Updating animal welfare thinking: moving beyond the “Five Freedoms” towards “a Life Worth Living”. *Animals* 6, 21.
- Mellor, D.J., 2017. Operational details of the five domains model and its key applications to the assessment and management of animal welfare. *Animals* 7, 60.
- Menchetti, L., Nanni Costa, L., Zappaterra, M., Padalino, B., 2021. Effects of reduced space allowance and heat stress on behavior and eye temperature in unweaned lambs: a pilot study. *Animals* 11, 3464.
- Mendl, M., Burman, O.H., Paul, E.S., 2010. An integrative and functional framework for the study of animal emotion and mood. *Proc. R. Soc. B: Biol. Sci.* 277, 2895–2904.
- Merriam-Webster. (n.d.). *Discomfort*. In Merriam-Webster.com dictionary. <https://www.merriam-webster.com/dictionary/discomfort> (Retrieved October 9, 2023).
- Millicamps, M., Tajerian, M., Naso, L., Sage, E.H., Stone, L.S., 2012. Lumbar intervertebral disc degeneration associated with axial and radiating low back pain in ageing SPARC-null mice. *Pain* 153, 1167–1179.
- Morton, D.B., Griffiths, P.H.M., 1985. Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. *Vet. Rec.* 116, 431–436.
- Moons, C.P.H., Ampe, B., Sonck, B., Vandaele, L., De Campeneere, S., Tuytens, F.A.M., 2015. Effect of summer conditions and shade on behavioural indicators of thermal discomfort in Holstein dairy and Belgian Blue beef cattle on pasture. *Animal* 9, 1536–1546.
- Moons, C.P.H., Sonck, B., Tuytens, F.A.M., 2014. Importance of outdoor shelter for cattle in temperate climates. *Livest. Sci.* 159, 87–101.
- Münster, M., Hörauf, A., Lübke-Becker, A., Grest, P., Rütten, M., 2013. Idiopathic esophagopathies resembling gastroesophageal reflux disease in dogs. *Tierarztl. Prax. Ausg.* 41, 137–139.
- Murphy, E., Melotti, L., Mendl, M., 2021. Assessing emotions in pigs: determining negative and positive mental states. Understanding the behaviour and improving the welfare of pigs 455–496.
- Murphy, E., Nordquist, R.E., van der Staay, F.J., 2014. A review of behavioural methods to study emotion and mood in pigs, *Sus scrofa*. *Appl. Anim. Behav. Sci.* 159, 9–28.
- Neethirajan, S., 2022. Affective state recognition in livestock—artificial intelligence approaches. *Animals* 12 (6), 759.
- Nicetto, T., Longo, F., 2019. Supercutaneous plating for the treatment of traumatic injuries of the appendicular skeleton in dogs. *Vet. Comp. Orthop. Traumatol.* 32, 149–157.
- Niittynen, T., Riihonen, V., Moscovice, L.R., Koski, S.E., 2022. Acute changes in oxytocin predict behavioural responses to foundation training in horses. *Appl. Anim. Behav. Sci.* 254, 105707.
- Olesen, J.E., Christensen, S., Jensen, P.R., Schultz, E., Rasmussen, C., Kjer, K.H., ... Henriksen, L. (2021). *AgriFoodTure: roadmap for sustainable transformation of the Danish agri-food system*.
- Olsson, I.A.S., J. Nicol, C., Niemi, S.M., Sandøe, P., 2020. From unpleasant to unbearable—Why and how to implement an upper limit to pain and other forms of suffering in research with animals. *ILAR J.* 60, 404–414.
- Ouahrani-Bettache, S., Jiménez De Bagüés, M.P., De La Garza, J., Freddi, L., Bueso, J.P., Lyonais, S., Occhialini, A., 2019. Lethality of *Brucella microti* in a murine model of infection depends on the wbkE gene involved in O-polysaccharide synthesis. *Virulence* 10, 868–878.
- Panteleeva, S., Reznikova, Z., Vygonayilova, O., 2013. Quantity judgments in the context of risk/reward decision making in striped field mice: first “count,” then hunt. *Front. Psychol.* 4, 53.
- Paul, E.S., Harding, E.J., Mendl, M., 2005. Measuring emotional processes in animals: the utility of a cognitive approach. *Neurosci. Biobehav. Rev.* 29, 469–491.
- Paul, E.S., Mendl, M.T., 2018. Animal emotion: descriptive and prescriptive definitions and their implications for a comparative perspective. *Appl. Anim. Behav. Sci.* 205, 202–209.
- Peana, I., Francesconi, A.H.D., Dimauro, C., Cannas, A., Sitzia, M., 2017. Effect of winter and spring meteorological conditions on milk production of grazing dairy sheep in the Mediterranean environment. *Small Rumin. Res.* 153, 194–208.
- Petit, J.Y., Doré, V., Marignac, G., Perrot, S., 2017. Assessment of ocular discomfort caused by 5 shampoos using the Slug Mucosal Irritation test. *Toxicol. In Vitro* 40, 243–247.
- Piccolo, F.L., Busch, K., Palić, J., Geisen, V., Hartmann, K., Unterer, S., 2019. Toxoplasma gondii-associated cholecystitis in a cat receiving immunosuppressive treatment. *Tierarztl. Prax. Ausg. K Klientiere Heimtiere* 47, 453–457.
- PIGWEB. (2024). *Policy brief: harmonization of pig research*. Retrieved from: <https://www.pigweb.eu/policypapers>.
- Pigatto, J.A.T., Borges de Vargas, E.V., Torikachvili, M., de Albuquerque, L., Caldart Andrade, M.C., dos Santos, E.O., Driemeier, D., 2018. Conjunctival melanoma in a horse treated by tumor resection and cryotherapy. *Acta Sci. Vet.* 46, 258.
- Pilz, M., Fischer-Tenhagen, C., Thiele, G., Tinge, H., Lotz, F., Heuwieser, W., 2012. Behavioural reactions before and during vaginal examination in dairy cows. *Appl. Anim. Behav. Sci.* 138, 18–27.
- Pinna, C., Nannoni, E., Rigoni, G., Grandi, M., Vecchiato, C.G., Spiezio, C., Biagi, G., 2015. Effects of yogurt dietary supplementation on the intestinal ecosystem of a population of Emperor tamarins (*Saguinus imperator*). *Prog. Nutr.* 17, 231–237.
- Plesch, G., Broekens, N., Laister, S., Winckler, C., Knierim, U., 2010. Reliability and feasibility of selected measures concerning resting behaviour for the on-farm welfare assessment in dairy cows. *Appl. Anim. Behav. Sci.* 126, 19–26.
- Polsky, L., von Keyserlingk, M.A., 2017. Invited review: effects of heat stress on dairy cattle welfare. *J. Dairy Sci.* 100, 8645–8657.
- Post, I.C., Dirkes, M.C., Heger, M., van Loon, J.P., Swildens, B., Huijzer, G.M., van Gulik, T.M., 2012. Appraisal of the porcine kidney autotransplantation model. *Front. Biosci. - Elite* 4, 1345–1357.
- Price, J., Nolan, A.M., 2001. Analgesia of newborn lambs before castration and tail docking with rubber rings. *Vet. Rec.* 149, 321–324.
- Raja, S.N., Carr, D.B., Cohen, M., Finnerup, N.B., Flor, H., Gibson, S., Keefe, F.J., Mogil, J. S., Ringkamp, M., Sluka, K.A., Song, X.J., 2020. The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises. *Pain* 161, 1976–1982.
- Rault, J.-L., Lay, D.C., Marchant-Forde, J.N., 2011. Castration induced pain in pigs and other livestock. *Appl. Anim. Behav. Sci.* 135, 214–255.
- Richards, D., Gever, J.R., Ford, A.P., Fountain, S.J., 2019. Action of MK-7264 (gefapixant) at human P2X3 and P2X2/3 receptors and in vivo efficacy in models of sensitisation. *Br. J. Pharmacol.* 176, 2279–2291.
- Ridner, S.H., 2004. Psychological distress: concept analysis. *J. Adv. Nurs.* 45, 536–545.
- Rix, A., Drude, N., Mrugalla, A., Mottaghy, F.M., Tolba, R.H., Kiessling, F., 2020. Performance of severity parameters to detect chemotherapy-induced pain and distress in mice. *Lab. Anim.* 54, 452–460.
- Russell, J.A., Bachorowski, J.A., Fernández-Dols, J.M., 2003. Facial and vocal expressions of emotion. *Annu. Rev. Psychol.* 54, 329–349.
- Schlicht, E., Kempenaers, B., 2018. The immediate impact of ringing, blood sampling and PIT-tag implanting on the behaviour of Blue Tits *Cyanistes caeruleus*. *Ardea* 106, 39–98.
- Schwartzkopf-Genswein, K.S., Stookey, J.M., Passillé, A.D., Rushen, J., 1997. Comparison of hot-iron and freeze branding on cortisol levels and pain sensitivity in beef cattle. *Can. J. Anim. Sci.* 77, 369–374.
- Sneddon, L.U., 2015. Pain in aquatic animals. *J. Exp. Biol.* 218, 967–976.
- Sneddon, L.U., Braithwaite, V.A., Gentle, M.J., 2003. Do fishes have nociceptors? Evidence for the evolution of a vertebrate sensory system. *Proc. Roy. Soc. London, Ser. B, Biol. Sci.* 270, 1115–1121.

- Stefanowska, J., Plavsic, M., Ipema, A.H., Hendriks, M.M.W.B., 2000. The effect of omitted milking on the behaviour of cows in the context of cluster attachment failure during automatic milking. *Appl. Anim. Behav. Sci.* 67, 277–291.
- Swan, J., Boyer, S., Westlund, K., Bengtsson, C., Nordahl, G., Törnqvist, E., 2023. Decreased levels of discomfort in repeatedly handled mice during experimental procedures, assessed by facial expressions. *Front. Behav. Neurosci.* 17, 1109886.
- Swartz, T.H., Bryant, D.M., Schramm, H.H., Duncan, A.J., White, R.R., Wood, C.M., Petersson-Wolfe, C.S., 2023. The effects of dexamethasone administration on physiological, behavioral, and production parameters in dairy cows after a difficult calving. *J. Dairy Sci.* 106, 653–663.
- Tannenbaum, J., Bennett, B.T., 2015. Russell and Burch's 3Rs then and now: the need for clarity in definition and purpose. *J. Am. Assoc. Lab. Anim. Sci.* 54, 120–132.
- Tate, T., Pearlman, R., 2019. What we mean when we talk about suffering—And why Eric Cassell should not have the last word. *Perspect. Biol. Med.* 62, 95–110.
- Tétreault, P., Dansereau, M.A., Doré-Savard, L., Beaudet, N., Sarret, P., 2011. Weight bearing evaluation in inflammatory, neuropathic and cancer chronic pain in freely moving rats. *Physiol. Behav.* 104, 495–502.
- Underwood, W.J., McGlone, J.J., Swanson, J., Anderson, K.A., Anthony, R. (2013). **Agricultural animal welfare. In *Laboratory Animal Welfare*, 233–278, London.**
- Upchurch, D.A., Renberg, W.C., Roush, J.K., Milliken, G.A., Weiss, M.L., 2016. Effects of administration of adipose-derived stromal vascular fraction and platelet-rich plasma to dogs with osteoarthritis of the hip joints. *Am. J. Vet. Res.* 77, 940–951.
- Van Beirendonck, S., Driessen, B., Geers, R., 2011. Painful standard management practices with piglets: does bundling and/or anesthesia improve animal welfare? *J. Clin. Anesth. Clinical Res.* 2, 157.
- Van der Heide, M.E., Stødkilde, L., Værum Nørgaard, J., Studnitz, M., 2021. The potential of locally-sourced european protein sources for organic monogastric production: a review of forage crop extracts, seaweed, starfish, mussel, and insects. *Sustainability.* 13, 2303.
- Van Zeeland, Y., Schoemaker, N., 2023. Pain recognition in ferrets. *Vet. Clin.* 26, 229–243.
- Vicario-de-la-Torre, M., Caballo-González, M., Vico, E., Morales-Fernández, L., Arriola-Villalobos, P., De Las Heras, B., Molina-Martínez, I.T., 2018. Novel nano-liposome formulation for dry eyes with components similar to the precocular tear film. *PPolymers.* 10, 425.
- von Borstel, U.U., Duncan, I.J.H., Shoveller, A.K., Merckes, K., Keeling, L.J., Millman, S. T., 2009. Impact of riding in a coercively obtained Rollkur posture on welfare and fear of performance horses. *Appl. Anim. Behav. Sci.* 116, 228–236.
- Walker, L.O., Avant, K.C., 2005. *Strategies For Theory Construction in Nursing*, 4. Pearson/Prentice Hall, Upper Saddle River, NJ.
- Wang, Y., Sun, D., Han, L., Wang, M., 2021. Bionic measurement of defecation in a swine model. *Physiol. Meas.* 42, 024003.
- Wemelsfelder, F., Haskell, M., Mendl, M.T., Calvert, S., Lawrence, A.B., 2000. Diversity of behaviour during novel object tests is reduced in pigs housed in substrate-impooverished conditions. *Anim. Behav.* 60, 385–394.
- Williams, A.M., Irurita, V.F., 2006. Emotional comfort: the patient's perspective of a therapeutic context. *Int. J. Nurs. Stud.* 43, 405–415.
- Whittaker, A.L., Howarth, G.S., 2014. Use of spontaneous behaviour measures to assess pain in laboratory rats and mice: how are we progressing? *Appl. Anim. Behav. Sci.* 151, 1–12.
- Zhang, M., Li, X., Li, J.H., Sun, H., 2017. Effects of confinement on physiological and psychological responses and expression of interleukin 6 and brain derived neurotrophic factor mRNA in primiparous and multiparous weaning sows. *Asian-Australian J. Anim. Sci.* 30, 1350–1357.