frontiers | Research Topics

Reviews in animal welfare

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Published in
Frontiers in Veterinary Science

ISSN 1664-8714
ISBN 978-2-8325-5519-4
DOI 10.3389/978-2-8325-5519-4

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Reviews in animal welfare

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Citation

Padalino, B., Narayan, E., eds. (2024). *Reviews in animal welfare*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-5519-4

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frontiers | Frontiers in Veterinary Science

TYPE Editorial
PUBLISHED 23 September 2024
DOI 10.3389/fvets.2024.1485518

OPEN ACCESS

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RECEIVED 23 August 2024
ACCEPTED 05 September 2024
PUBLISHED 23 September 2024

CITATION
Narayan E and Padalino B (2024) Editorial: Reviews in animal welfare.
Front. Vet. Sci. 11:1485518.
doi: 10.3389/fvets.2024.1485518

Editorial: Reviews in animal welfare

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KEYWORDS

animal welfare, farming and wildlife, health, food, Five Domains Model

Editorial on the Research Topic
Reviews in animal welfare

During the past decade, the term "welfare" has been widely discussed in animal industries. The growing number of wildlife-human conflicts caused by urban expansion and climate change reveals the urgent need of solutions that can ensure both wildlife welfare and human development requirements. In terms of farm animals, with the promotion of intensive production, whether intensification is leading to the suffering and poor welfare of animals has been a highly debated Research Topic. Pet species such as cats and dogs also received widespread welfare concerns to improve their quality of life. However, some issues related to animal welfare have been studied less in depth than others, more research is warranted to understand animal welfare issues across animal production systems and to come up with innovative solutions to improve animal health and wellbeing.

In this edition, we focussed on discussing our current knowledge of animal welfare. This Research Topic invited reviews to demonstrate available technologies, assessment tools, and methodologies that can be used to quantify and monitor animal welfare. Papers also explored the quality and validity of animal welfare related scientific research design and writing. Emerging Research Topics such as positive welfare was also covered in this Research Topic.

We collected 10 reviews (and one research article) on animal welfare Research Topics, related to a variety of species, from companion animals to livestock, and from laboratory animals to wild animals. The papers identified current gaps of knowledge and recent advancements in the field of animal welfare.

The first manuscript by Adamakopoulou et al. demonstrated how "cats and dogs" related welfare literature has evolved worldwide over the last 40 years. The authors described the main research interests and discussed gaps in knowledge by conducting a search using Scopus® and applying an innovative text mining approach. The study included a total of 1,775 scientific literature records that probed into cats' and dogs' welfare aspects. Descriptive statistical results, which were based on titles and abstracts of the records showed an increasing number of studies on this Research Topic while researchers in Europe and North America showed strong interest in cat and dog welfare research. Keywords such as "behavior," "owner," and "adopt" were frequently mentioned with "shelter" being the most used word in literature records. Some of the other keywords identified in this review included "stress and housing conditions," "welfare and pain assessment," "shelter management," "euthanasia," and "owners" and veterinarians' perceptions' of cats and dogs welfare.

In the second Research Topic, Masebo et al. analyzed the welfare of camels. Specifically, the authors studied intensive management system and regulations by reviewing 43 years of main Research Topics on camel welfare. The researchers suggested that over this period, attention has been raised to camel welfare with Asian authors contributing greatly to academic research. Among the 234 literature records analyzed in this study, "milk," "calve," "behavior," "female," "breed," and "stable" were notable keywords. Beginning with the oldest Research Topics such as "female and male reproduction," researchers over time covered a wide range of Research Topics like calf management, milk production, health and management system, behavior, and feeding.

The third paper by Littlewood et al. provided introduction to the concept of agency, and discussed the relationship between agency and positive emotions, experience, and welfare. The discussions were based on The Five Domains Model, which was updated in 2020 and redefined the Domain 4 from "Behavior" to "Behavioral Interactions." The authors illustrated how the renamed domain can be used to evaluate positive animal welfare and took captive sugar gliders and racing greyhounds as real-life examples to demonstrate the applications of this domain.

The fourth paper was from the researchers Yan et al. who conducted qualitative evaluation of laboratory animal welfare among a total of 150 undergraduate and 148 postgraduate veterinary medicine students in China. The results given by respondents showed an overall strong sense of responsibility among students who were engaged in animal experiments, laboratory teaching, and learning to support and contribute to the improvement of laboratory animal welfare. However, the authors also pointed out that despite the passion, basic theoretical knowledge of animal ethics, adequate compass of experimental techniques and awareness of the existence of related supervisory agencies were still insufficient among current students. This study aimed to lay the cornerstone for the future of veterinary education and the development of humanity, compassion, as well as professional skills of veterinary students.

The fifth study by Whitham and Miller reviewed the existing evidence of affective state related vocal production in non-human mammals, and current available non-invasive methods used to investigate vocal activities. The authors highlighted that apart from negative contexts measurements such as pain levels and social isolation, acoustic activity can also be utilized as an indicator of positive affective state. Such vocalization is produced when animals are foraging, playing, grooming, or interacting with their intimate fellows. Related to cardiac activity, respiration rates, and hypothalamus-pituitary adrenal (HPA) axis activity, vocalization can be an effective tool for wildlife scientists to identify welfare-associated events. Vocalization examination discussed in this study could be achieved by applying modern acoustic monitoring systems and were valuable for practical situations such as husbandry routine or environment management, animal transfers, and introductions to monitor animal welfare and quality of life.

The sixth manuscript by Ghimire et al. focused on a controversial Research Topic- the welfare of the Asian elephants *(Elephas maximus)* that were involved in religious activities and in the logging or tourist industries. In this paper, the authors reviewed a variety of available animal welfare assessment tools (e.g., ZooMonitor, WelfareTrack, ZIMS, etc.), with a special emphasis on elephants (Elephant Behavioral Welfare Assessment Tool, Elephant Welfare Initiative, etc.). The toolkit includes methodologies based on various resources such as digital information systems, paper-based work, keeper ratings, welfare grading scales, etc. However, considering the multiplex captive environments of elephants throughout Asia, the authors also pointed out that further development of a comprehensive and practical tool is necessary.

The seventh paper by Sundman et al. investigated the welfare issue related to ill and injured feedlot cattle. In this study, the authors reviewed a total of 110 articles, in which only 12 articles mentioned the management of ill and injured cattle in specialized hospitals and two discussed the application of chronic pens. Although diagnoses such as Bovine Respiratory Disease Complex (BRDC), lameness, and gastrointestinal problems are very common in feedlots, these results indicated the current knowledge gaps in the negative valence of animal welfare caused by illness and injury in feedlot cattle. Sundman et al. also explored the potential of the Five Domains Model in the individual management of sick cattle, aiming at strengthening the welfare assurance in current industry practices and assisting producers toward understanding their animals' behavioral needs.

In the eighth study, Neves et al. probed into the causes of unsatisfying reproducibility in scientific research that require animal experiments. The authors noted that their review of 124 journal articles suggests that low quality and transparency of scientific writing, such as deficiency in data specification and description of crucial details, are the main causes of irreproducibility. The articles evaluated showed an assured demand for practical application of the 3R's principle (Refine, Reduce, and Replace) and international guidelines in experiment design, especially in areas such as metabolism, immunity, hormones, and stress to further improve the overall welfare and reduce the number of laboratory animals required in experimental protocols, avoid unnecessary financial input, and at the same time, alleviate compassion fatigue found in veterinarians and laboratory animal technicians.

The ninth review by Linstädt et al. covered a total of 2,818 English and German publications from 2011 to 2021 in five databases. This study systematically discussed the reliability of various welfare evaluation methods that can be applied in different environments such as farming systems and pasture-based systems. The authors summarized the validity of stress monitoring parameters such as hair cortisol concentration, heart rate variability, as well as biomarker research and behavioral studies. The authors also discussed emerging tools such as Precision Livestock Farming and Animal Need Index and Herd Data, aiming at improving the efficiency of livestock managers in evaluating animal welfare and identifying potential welfare concerns. At the end of this paper, Linstädt et al. recommended that importance should be attached to easily observable indicators such as lameness and body condition score in welfare assessment.

In the 10th study, Rokade et al. investigated the attitude of egg producers in India toward the global shift from the traditional battery cage poultry production system to the cage-free egg production system. While deficiencies such as limited space for movement in the cage, and inappropriate flooring can commonly

be found in battery cage systems, cage-free systems are now regarded as a less cruel type of system, providing better welfare to the hens. This needs-assessment survey also highlighted producers' requests for corresponding support like financial assistance and technical training from both government and privates, to better cope with the building and development of the cage-free sector and be able to compete with battery cage poultry producers in the market.

In the 11th and final Research Topic, Fox et al. used 70 topknot wool samples in non-invasive cortisol and testosterone assessment to quantify the stress levels that rams were experiencing in Queensland, Australia. The authors also analyzed the potential relationship between the two different hormones. As an important indicator of stress response in animals, cortisol levels studied in this research provided important information on the quality of husbandry management and explore the large margin to improve animal welfare in the sustainable sheep industry. In recent years, non-invasive hormone assessment has been utilized by producers and researchers as an effective tool to assess long-term, historic reflections of stress levels, without being affected by acute stressors as sampling at the time of collection.

Overall, this Research Topic highlights some of the recent developments in the broad field of animal welfare assessment in different species.

Author contributions

EN: Writing – original draft, Writing – review & editing. BP: Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

The editor thanks the Frontiers admin team for their wonderful support with the Research Topic as well as the numerous peer reviewers.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

frontiers | Frontiers in Veterinary Science

TYPE Review
PUBLISHED 12 October 2023
DOI 10.3389/fvets.2023.1268821

OPEN ACCESS

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RECEIVED 28 July 2023
ACCEPTED 29 September 2023
PUBLISHED 12 October 2023

CITATION
Adamakopoulou C, Benedetti B, Zappaterra M, Felici M, Masebo NT, Previti A, Passantino A and Padalino B (2023) Cats' and dogs' welfare: text mining and topics modeling analysis of the scientific literature.
Front. Vet. Sci. 10:1268821.
doi: 10.3389/fvets.2023.1268821

Cats' and dogs' welfare: text mining and topics modeling analysis of the scientific literature

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Animal welfare is a field with increasing significance and has been raising huge concerns of the public and the political stage. Cats and dogs possess an important role in human life, but their welfare is not always secured from a legal aspect. This review aimed to describe the evolution and geographical distribution of "cats and dogs" and "puppies and kittens" welfare literature over the last 40 years, distinguish the main research topics studied and highlight gaps in knowledge. A search using Scopus® was performed with different search strings and predetermined filters as time range, language, and subject area. A total of 2,725 scientific literature records were retrieved but only the ones that referred to cats and dogs' welfare aspects were retained. The final 1,775 records were processed through descriptive statistics, and text mining and topic analysis procedures were performed on their titles and abstracts. The results showed that the number of studies has been increasing, especially in Europe and North America. "Shelter" was the most frequent word, followed by "behavior," "owner" and "adopt." The nine topics that emerged from the analysis were breeding, stress and housing conditions, welfare and pain assessment, public health, shelter management and euthanasia, behavioral problems, health issues and management, human-animal interaction, and owners' and veterinarians' perceptions. While stress and housing conditions, public health, and owners' and veterinarians' perceptions were the most studied topics, human-animal interaction was the least studied. This review confirmed the increasing research and interest in cats' and dogs' welfare and showed gaps in knowledge where further studies are needed.

KEYWORDS

companion animals, canine, feline, well-being, behavior, systematic review, machine learning

1. Introduction

Public concerns regarding the ethical treatment of animals have been growing over the years. This increased interest has led to the establishment and development of animal welfare science (1). According to Broom (2) the definition of welfare states 'The welfare of an individual is its state as regards its attempts to cope with its environment.'. Animal welfare science can be characterized as one of the most complicated and inclusive fields in biology (3). It raises a variety of concerns that involve the fundamentals of life such as freedom from pain and injury, water and food supply, and shelter. These concerns can be grouped into three main headings that focus on proper biological function, balanced emotional state, and expression of natural

behavior (4). An animal must be in good physical and mental health status to reach a balanced emotional state (5).

Animal welfare has been a subject of political interest for several decades (6, 7). Legislation on livestock animal welfare involves a highly strict framework on welfare on-farm, during transportation and slaughter (8) and they are often based on research-based evidence (9–12). On the contrary, in the case of cats and dogs, regulation worldwide has been slower to develop, both in terms of topics covered and specificity. In regions such as the United States and the South Wales State in Australia, legislation has the minimal requirements regarding dog and cat keeping and everyday handling. In fact, most of the time, the legislation is just between the lines of general anti-cruelty and animal welfare statutes (13). In Europe, the legislation only focuses on transportation and veterinary controls, making all the other aspects of pet welfare to be monitored by a national regulation system that differentiates from state to state (8). Cats and dogs hold a significant place in people's daily lives and are considered members of their families providing not only companionship but also serving as a source of affection and emotional attachment (14). Dogs have expanded their role from being companions to providing aid as guides and assistants for people with disabilities (15, 16). As pets play a crucial role in providing companionship, the changing human lifestyles and demands can harm their well-being (17). It seems easy to presume the idea that pets are treated with respect as companion animals and their "good welfare" is granted, but there is not much evidence to confirm this belief (18). In fact, despite this assumption, there remain significant concerns regarding pet welfare (18, 19). One of the biggest issues undermining the welfare of dogs and cats is pet overpopulation and the burden on animal shelters. In the US, more than 3.5 million entries in shelters (including both dogs and cats) were recorded in 2019 (20). In Europe, there is currently no official data on the number of dogs and cats in shelters, but it is estimated that there are about 100 million abandoned pets in total, including not only those in shelters but also stray dogs and cats (21). Stray dogs are a major problem in several areas of southern and eastern Europe and a major public health concern, increasing the risk of aggression toward humans and other livestock and the transmission of rabies (22). Another area of animal welfare concern is the breeding of brachycephalic breeds of dogs and cats with brachycephalic (shortened, flattened) head structures, which also raises ethical concerns due to the associated health problems (23, 24). Furthermore, recently there has been an alarming rise in pet obesity, primarily due to limited access to exercise and excessive food consumption, resulting in severe health issues (24). A significant number of dog owners mistakenly believe that an overweight body condition is ideal for their pets (25). On the other hand, some pet owners are interested in feeding a plant-based diet, but vegetarian and vegan diets have been considered contraindicated in cats (26, 27). Finally, when humans anthropomorphize animals, they attribute to them their traits, emotions, or intentions, and this attitude may compromise pet welfare too (28).

The need for mandatory legislation to establish common policies and address inadequate legal systems must therefore be undertaken immediately (29). Fortunately, new regulations to protect companion animals' welfare will be issued shortly in Europe (30) but there is a need for research-based evidence. Starting from these considerations, this review aimed to examine in detail this research field by using text mining and topic analysis techniques. The goal of text mining analysis is to identify the most important words within the text and topic modeling is a tool to uncover the structure of meaningful topics among collections of records as well as to discover hidden textual patterns (31). Through this analysis, this review seeks to extract valuable insights from a vast amount of scientific literature enabling the analysis of different topics within the field, tracking their evolution over time, and identifying any gaps in knowledge.

2. Materials and methods

2.1. Data Set

A literature search protocol using Scopus®, the abstracts and citation database of Elsevier©, was set up to identify the peer-reviewed records that covered the topic of "cats and dogs' welfare" and "puppies and kittens' welfare." The search was performed during May and June 2023. The research was refined based on the year of publication (from 1980 to 2023), scientific area (Veterinary and Agricultural and Biological Sciences topics), article type (review and scientific article), and language (English). The first search string, "cat OR dog AND welfare," retrieved 2,532 records, while the second search string, "puppy OR kitten AND welfare," retrieved 193 records. Setting these conditions, the produced records were 2,725. These records were inserted in an electronic Excel workbook (Microsoft Excel®, v16.0, Redmond, WA, United States) to perform further screening and analysis. The Excel spreadsheet organized the information in a tabular format, where each record was represented in a row, and the record's information was organized in columns. The information in each column included title, authors, affiliations, abstracts, year of publication, type of record (e.g., article or review), and source of publication (i.e., name of the journal). After that, the elimination of the duplicates was performed since the same records could have been included in both search strings conducted. Starting from the original 2,725 records, an automatic exclusion of the records that had no abstract available was then performed for the construction of the final spreadsheet. After that, a further manual exclusion was performed by the four reviewers (CA, MZ, MF, and BP). The criteria for the manual exclusion were wrong topics, such as records about human welfare that referred to how pet companionship increases the well-being of humans and records that focused on other species (e.g., cattle). During the latter screening, the scientific records were categorized based on the relevant species (i.e., dogs, cats, dogs, and cats). The final number of records included was 1,775. Results of the systematic scientific literature search and the subsequent automatic and manual screening of records are represented schematically in Figure 1. Descriptive statistics were performed on the selected records to profile the scientific corpus (i.e., authors, country of the corresponding author, title of the paper, abstract, year and journal of publication) based on information recorded from the Scopus® database. Descriptive statistics was also performed based on the species (i.e., dogs, cats, or both) included in the scientific literature records. Pivot tables were made to count the number of records per year and to highlight the most represented nationality and regions in the document corpus. The nationality of each document was derived based on the affiliation of the corresponding author/first author.

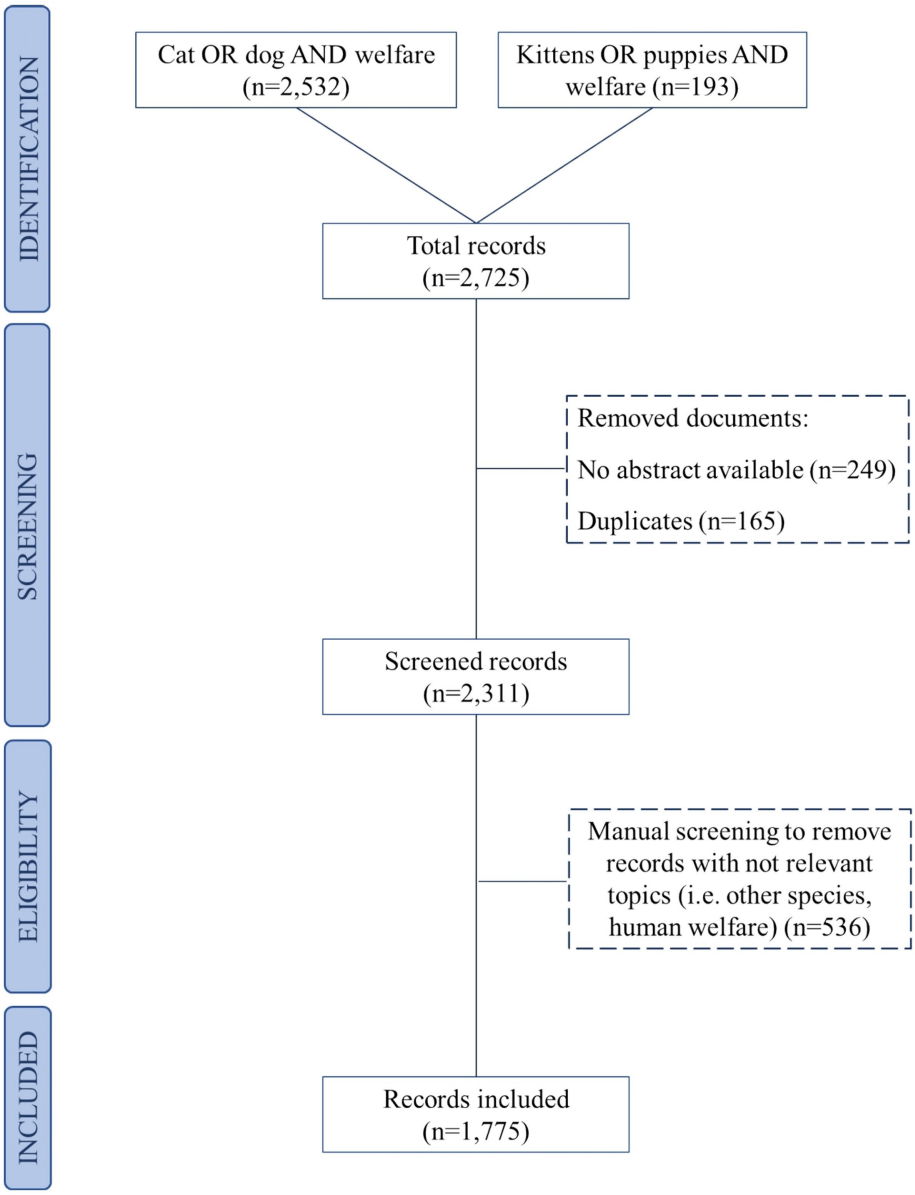

FIGURE 1
Flow chart representing scientific literature search and each step of selection of the scientific literature records on dogs' and cats' welfare. The number of the excluded records and their reason for exclusion from the study are represented by the dashed lines. Not-relevant species were every species different from dogs and cats.

2.2. Text mining

To conduct text mining analysis, a separate Excel sheet was created with two specific columns: "doc_id," with the progressive numeration of the 1,775 scientific literature records, and "text" which contained the abstracts of the retained records. Text mining analysis involved converting the text into a numerical representation to identify important patterns within the data corpus. Since some words in the corpus of records were spelt in both American and British English, the authors decided to standardize the corpus of documents using only British English. The text mining analysis was performed in the R studio environment using a combination of functions in the packages "tm," "snowballC," "ggplot2," "dplyr" and "tidyverse." Text mining was performed considering the titles and abstracts of the 1,775 records. The pre-processing steps that the researchers followed involved what was reported in the literature (32). Namely:

- Convert the text to lowercase: All the capital letters inside the corpus were converted into lowercase letters.
- Removal of strange symbols and fonts: Symbols and fonts such as "@," "/" or "*" were removed and replaced by white space.
- Removal of punctuations: Punctuations in the corpus were removed and replaced by white spaces.
- Exclusion of certain characters: punctuation, blanks, and numerical digits.

- Exclusion of "stop words." These frequently used words, while common in the language, do not provide specific information about the content of the document. In the case of this review the researchers decided to remove as stopwords the following words: "dog," "dogs," "cat," "significance," "significant," "significantly," "group," "groups, "test," "animal," "animals," "study," "studies," "cats," "welfare," "well-being," "research," "researches," "will," "control," "data," "different."
- Removal of numbers: Numbers were removed and replaced by white spaces.
- Removal of extra white spaces: Extra white spaces that occurred from previous steps were removed.
- The application of a stemming algorithm. This involves reducing words to their root forms, also known as tokenization, and helps to avoid counting the same word multiple times when it appears in different grammatical forms (e.g., "management" and "managerial" become "manag"). Stemming helps to standardize the representation of words and allows for a more accurate analysis of word frequencies and associations.

Afterward, a matrix was built containing along the rows and the terms along the columns, the so-called document-term matrix and a term frequency-inverse document frequency (TF-IDF) technique were used. The TF-IDF technique, employed to assign a relative weight to words (33), considers the frequency of a term within a document while considering how widely it is used across the entire collection of records. This adjustment reflects the importance of a word in the overall context of the document set. To identify the most important words, a threshold of TF-IDF value greater than or equal to 13 was used. These highly relevant words were then represented as a histogram, visually displaying their frequencies. Additionally, a word cloud was created.[1] In this word cloud, the size of each word is proportional to its TF-IDF value. A larger character size indicates a higher TF-IDF value, highlighting the words that are more significant in the collection of records. Associations among the most frequent words (TF-IDF ≥ 13) and all the corpus terms were determined. The grade of correlation was set ≥0.2 and associations were identified by measuring the frequency with which two words appear together. In particular, the correlation is 1 if two words are always together and −1 if they are never together.

2.3. Topic analysis

The approach used for the topic modeling analysis in this review was the Latent Dirichlet Allocation (LDA). It is a probabilistic model based on the intuition that a single topic can be described as a multinomial distribution of words and a single document can be described as a multinomial distribution of latent topics (34). The words used in the topic analysis were those contained in the titles and abstracts of the 1,775 scientific literature records after pre-processing and text mining steps. The LDA function was used with the Gibbs sampling option of the "topic models" package in R (35). The LDA function returns a list of objects, which was then passed to the function 'topics' to create a table where each record is matched with one of the topics. We decided *a priori* to look at 6 and 9 topics for the topic analysis, and with the consensus among the researchers, the most indicative was chosen. Then, the resulting topics were ranked according to the cumulative probability of the first 15 words of each topic. The individual topics were visualized in a bar histogram representation with the probabilities of the first 15 words within each topic (beta values) and the authors attributed a name to each topic as suggested in the literature (36).

3. Results

3.1. Descriptive statistics

The number of publications per year from 1980 to 1994 was fewer than 10 records, whereas there was a significant increase in the number of records from 2005 to the present year (Figure 2).

Nearly half of the identified records (47%) had corresponding or first authors from Europe, making it the most prominent region in terms of authorship. North America accounted for 30% of the records, making it the second most important region researching companion animal welfare topics. Oceania, Asia, South America, and Africa had progressively lower percentages of records, with 12, 5, 5, and 1%, respectively. The results are shown as a pie chart in Figure 3. Figure 4 shows instead the graph of European nations with the most records.

Dogs were the species with the highest number of records (1,031/1775, 58.1%). There were 455/1775 (25.6%) records for cats and 289/1775 (16.3%) records relating to both species.

3.2. Text mining

The most frequent words with a weight over 13 (TF-IDF ≥ 13) are shown as a histogram in Figure 5. A word cloud with the most frequent words is represented in Figure 6 in which the size of the font is proportional to the TF-IDF of every word. A correlation coefficient of 0.2 was discovered between the most important words (with a TF-IDF score of 13 or higher) and the remaining words in the matrix. These correlations are presented in Table 1. No significant correlation (with correlation grade ≥ 0.2) with other words was shown by the words "behavior," "human," "manag," "effect" and "report."

3.3. Topic analysis

Figure 7 shows the 9 topics with the attributed names, and their first 15 words. The most consistent topic was the one named "Stress and housing conditions" (topic 4) followed by topic 9 ("Public health"), and topic 3 ("Owners' and veterinarians' perceptions") with a number of records of 235, 228, and 226, respectively. Following closely behind were Topic 6 ("Health issues and management") with 208 records and Topic 2 ("Shelter management and euthanasia") with 201 records. Topic 8 ("Human-animal interaction") had the lowest number of records published, with only 132 records. The results of the trend analysis for the period 1980 to 2023 were represented in graphs for each topic in Figure 8. A trendline showed that for all the topics there was a significant increase in the number

1 www.wordclouds.com

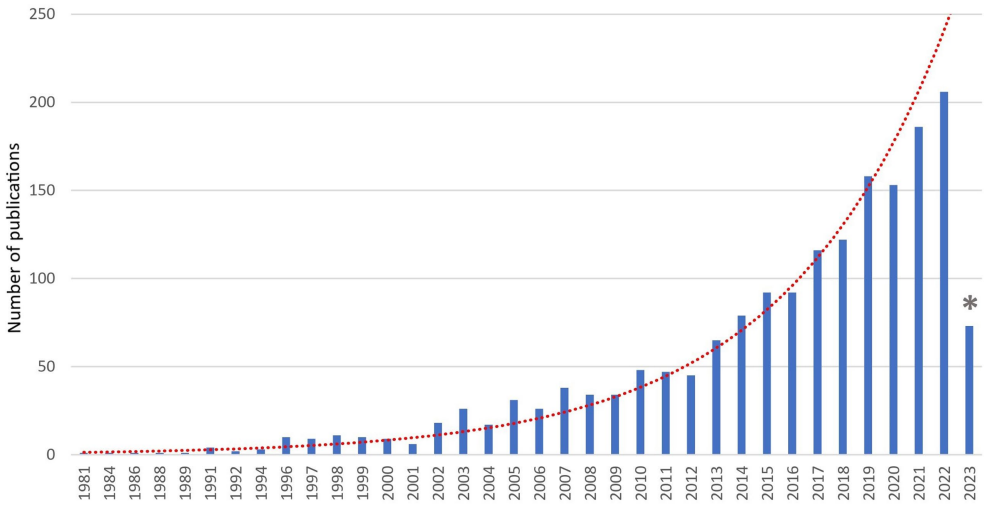

FIGURE 2
Peer-reviewed scientific literature records (n = 1,775) on the welfare of cats, dogs, puppies, and kittens from 1980 to 2023. The dashed line represents the trend over the years. The asterisk on the year 2023 indicates that results for that year are related to the period from January to March.

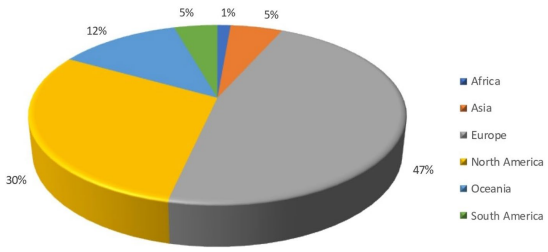

FIGURE 3
Pie chart depicting the distribution of the 1,775 scientific literature records selected for inclusion per regions and subregions, represented by their respective percentages.

of records, especially after 2010. Most of the records for every topic were published between the years 2019–2022. According to the graphs, topic 6 showed a steady trend in the number of published records from the year 2017–2022.

4. Discussions

In this study, we applied text mining analysis and topic modeling to extract valuable insight from a vast body of scientific literature allowing us to examine various topics in the field of companion animal welfare and identify any gaps in the current knowledge. Despite reaching some consensus on an applied definition of welfare, ongoing academic debate continues. Additionally, the political relevance of animal welfare science is strongly based on societal concerns regarding how animals are treated. Animal welfare is seen as a 'new science' by many and the development of companion animal welfare science is considered even newer (37). The application of text mining and topic analysis techniques to the cats' and dogs' welfare literature has therefore enabled a deep analysis of the research conducted over the past 40 years enhancing our understanding of the subject. The topics that emerged from the topic analysis were expected, particularly those relating to behavioral problems, housing conditions, and health, since those are the three areas that are well-developed in veterinary medicine and husbandry. However, topics such as human-animal interaction and the perception of welfare by owners and veterinarians also surfaced in recent years of research, reflecting the important role of humans and their relationship with animals in the concept of animal welfare. The findings obtained using this machine learning technique confirm also the multidisciplinary of animal welfare topics.

There has been an augmented increase in the number of records in the last 40 years, particularly after the 2000s. The lower number of records found in 2023 is because the search was carried out in May 2023, so the number of records published online was still limited, but an upward trend is expected. This upward trend of publication reflects the development of animal welfare science. The term animal welfare began to be used in 1947 (38), but it was only in the 1990s that it started to be considered a measurable scientific term (39). As it was stated above animal welfare on farm animals has been stricter and more well-regulated than in the case of cats and dogs but despite the delay, it became imperative to ensure the care of dogs and cats in a more secure legal framework (24). Since the early 2000s, countries such as Germany, Austria, and the United Kingdom began to strengthen their state legislation with laws that further shielded the protection of pets (29). In 2004, Italy introduced "Law 189," which aimed to prevent the abuse of animals and specifically addressed their involvement in underground fights or unapproved contests. Regulation must be based on research, so more funds for animal welfare were available generating more publications. As expected from the descriptive statistics, Europe was the dominant region with the United Kingdom, Italy, Spain, Germany, and Austria as the major geographical areas of origin for records on cats' and dogs' welfare. This reflects the region's pioneering and driving role in the field of animal protection and welfare promotion. North America followed in terms of the number of published records, and it is reasonable considering the large population and research opportunities, especially in the

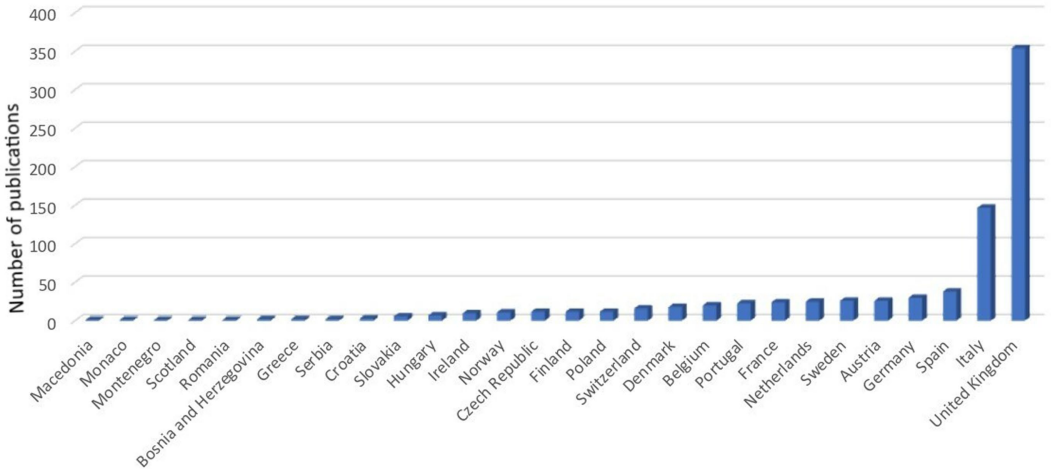

FIGURE 4
Distribution by European country of peer-reviewed scientific literature records (n = 1,775) on the welfare of cats, dogs, puppies, and kittens based on the nationality of the corresponding/first authors that are published from 1980 to 2023.

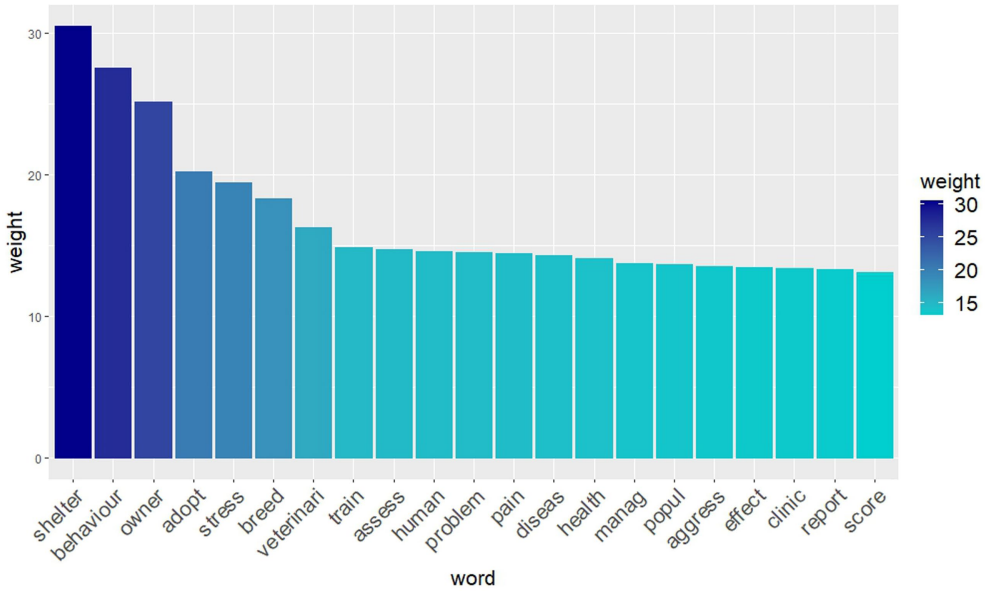

FIGURE 5
Histogram illustrating the most frequent words (i.e., words with term frequency-inverse document frequency (TF-IDF) values ≥13) and the weight of the 1,775 records included in the study.

United States where legislation is weak and many pet welfare issues have been highlighted (13).

The most frequent words were "shelter," "behavior," "owner," "adopt" and "stress" (with higher TF-IDF weight). The word "shelter," as the most frequent word, is not surprising as it may reflect the major problems of relinquishment and overpopulation of shelter animals that compromise the overall welfare of cats and dogs (40–42). It is also not a coincidence that the word "shelter" was associated with euthanasia. In fact, when animals are relinquished, they may be reclaimed, adopted, remain in shelters until they die, or be euthanized (43). Animal welfare groups are striving to reduce euthanasia rates, and many shelters around the world have adopted no-kill policies for adoptable animals as part of their mission. In countries, such as Italy, where a no-kill policy is in place, animals can remain in shelters until they die naturally, and euthanasia can only be used for dogs and cats that are dangerous or have a terminal illness or a health condition that makes life painful (44, 45). A no-kill policy can also have negative aspects. It can be costly and space intensive, leading to chronic overcrowding in shelters and compromising welfare standards. This is why other countries, such as the United States, use euthanasia. The decision to euthanize a shelter animal is influenced by a number of factors, including the animal's health and any behavioral problems (46). In addition, many shelters face the harsh reality of limited space and funding, which often forces them to make the

FIGURE 6
Word cloud with the most frequent words (i.e., words with term frequency-inverse document frequency (TF-IDF) values ≥13) of the 1,775 records included in the study. The words with larger font are the ones with higher weight.

difficult decision to euthanize animals in order to accommodate new arrivals (47). According to Shelter Animals Count database, a total of 325,301 cats and dogs were euthanized in the US shelters in 2019, with euthanasia being the final outcome for the 11.5% of cats and 6.9% of dogs (20). After 2019, the number of animals relinquished decreased by 16%, although the COVID19 pandemic led to a slight increase in the number of abandoned dogs and cats in 2022 compared to 2020 (48). Interestingly, factors such as age group and coat color have been found to play a role in shelter dog euthanasia decisions (49). The term "behavior" was quite frequent as animal "behavior" has been extensively studied and analyzed in various situations within the field of welfare, especially as a parameter of assessment (50–52). The words "owner" and "adopt" had also a higher probability because they can both be linked with the word shelter. The word "owner" comprises the perspective of the human in relation to the well-being of their companion animal and how they engage and interact with it. Finally, it is not surprising that the word "stress" was often used in the literature, as for a while stress-related responses have been used as indicators of poor quality of welfare.

The LDA analysis has identified nine different topics highlighting the diverse aspects of welfare, ranging from health to behavioral problems, pain, and management. This involves studying and connecting the different biological components, including physical and psychological factors, that together determine the level of welfare. This amplifies that the approach to welfare encompasses multiple disciplines and that the concept of welfare itself is broad and challenging to categorize (53). The topics that emerged align well with the four principles of "Good Feeding," "Good Housing," "Good Health," and "Appropriate Behavior" outlined in the Welfare Quality

TABLE 1 Correlation between the most relevant words (i.e., words with term frequency-inverse document frequency (TF-IDF) values ≥13) and the other words present in the corpus.

Words (TF-IDF ≥ 13)	Associated words (correlation ≥0.2)
Assess	tool (0.35), valid (0.34), reliabl (0.33)
Breed	pedigree (0.30), brachycephal (0.28), terrier (0.25), club (0.25), select (0.25), breeder (0.23), genet (0.22), bull (0.21), phenotyp (0.21), inherit (0.20)
Pain	analgesia (0.41), analges (0.34), scale (0.33), acut (0.31), chronic (0.22), challeng (0.21), advanc (0.20)
Shelter	enter (0.33), intak (0.24), stay (0.23), euthanasia (0.21), euthan (0.20)
Train	reinforc (0.43), trainer (0.35), punish (0.28), method (0.26), obedi (0.26), learn (0.23)
Adopt	return (0.26), characterist (0.25), color (0.21), coat (0.21), length (0.20)
Disease	infecti (0.24), preval (0.24), transmiss (0.24), infect (0.23), diagnosi (0.20)
Health	public (0.22)
Owner	questionnaire (0.26)
Problem	excess (0.26), destruct (0.21)
Stress	level (0.30), cortisol (0.25), stressor (0.21)
Veterinarian	surgeon (0.30), care (0.26), veterinarian (0.24), patient (0.23), client (0.21), medicin (0.21), practic (0.21), practition (0.21), nurs (0.20)
Agress	toward (0.30), fear (0.24)
Clinic	sign (0.27)
Popul	freeroam (0.34), dynam (0.27), densiti (0.22), roam (0.21), capac (0.20)
Score	qualiti (0.23), centr (0.20)

The grade of correlation was set ≥0.2.

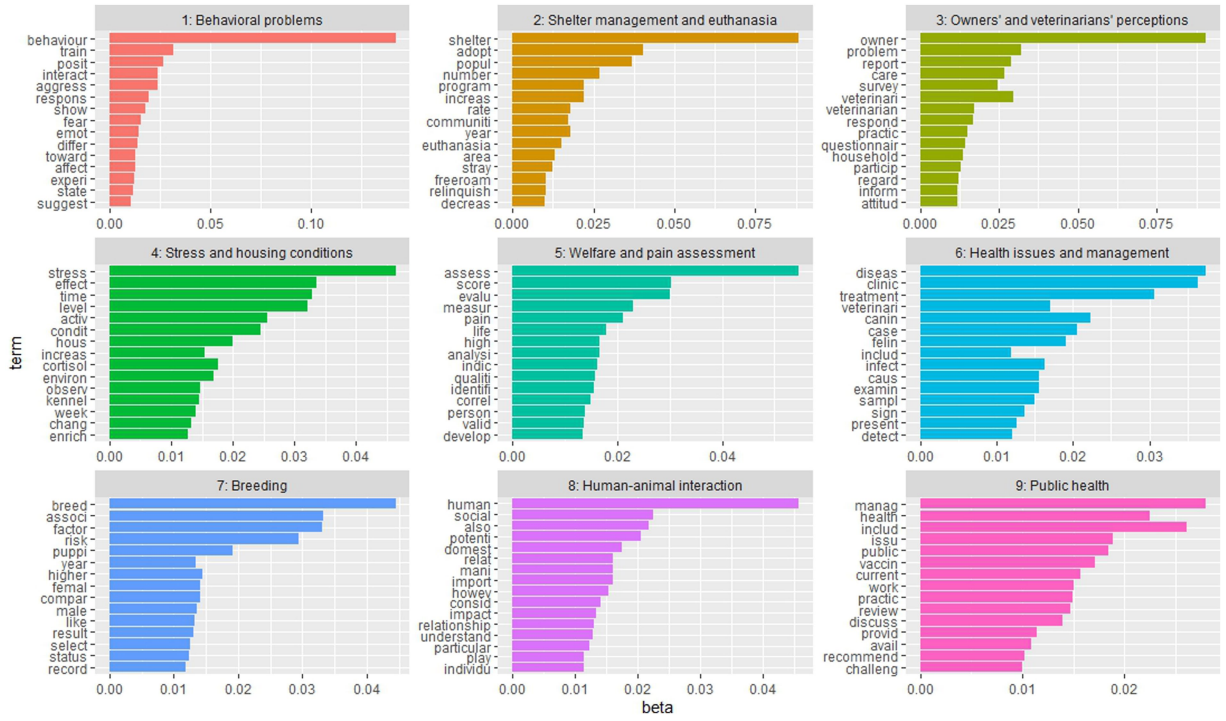

FIGURE 7
Histograms with the most frequent 15 words (terms) for the 9 topics of the 1,775 scientific literature records included in the study. The "beta" indicates the relative probability of each word belonging to each topic.

protocol (54). Topics 2 and 4 can be classified under the principle of "Good Housing" while topics 5, 6, 7, and 9 fall under the category of "Good Health." Topics 1, 3, and 8 can be associated with the principle of "Appropriate Behavior." It is worth noting that no specific topic was found to directly address the concept of "Good Feeding." Clearly, some aspects have been studied more than others and some of them earlier than others, reflecting also whether this argument has been seen as an aspect possibly related to welfare by the authors, which have used in their text the word "welfare."

As previously mentioned, the topic named "Stress and housing conditions" (topic 4) was that one with more scientific literature records. Stress has indeed gained significant importance as a topic due to its pervasive impact on welfare and this topic comprises the effect of housing conditions under the influence of the "good housing" principle in the stress behavior of cats and dogs. Terms such as "environmental enrichment" and "cortisol levels" are often observed in the research studies which entered this topic. It has been shown that an unenriched environment and inappropriate management by staff can lead to a low quality of life and compromise the welfare of dogs sheltered for long period (50). The second topic for number of records was "Public health" (topic 9). It was noted that this topic encloses subjects such as vaccination and management and represents a very well-researched topic through the years. Indeed, within 20 years, vaccination became an act of veterinary science that should be considered an individualized medicine, adjusted to the needs of each pet. Vaccination has been provided as preventive medicine, being part of an annual health check-up visit (55), and was also as a tool to preserve public health from zoonoses such as leptospirosis and rabies, which are common in stray dogs (56–58). Public health recently also raises concerns about the population of free-roaming animals and the overcrowded spaces in shelters (59, 60). In fact, neglected zoonotic diseases such as rabies and echinococcosis are transmitted at the stray-dog-human interface, particularly in low to middle-income countries (58). Another topic that was found important and is contained in the "Good Housing" principle and also related to public health is the topic named "Shelter management and euthanasia" (topic 2). As mentioned above, the shelter population of cats and dogs is rapidly increasing. This statement can be justified by the words "population," "number" and "increase" which were found to be associated with the current topic. The studies contained in this topic could also be considered under the umbrella of "one health" and "one welfare" approaches. It is indeed important to rise the welfare of the animals to also enhance human and planet well-being.

As expected, numerous published records, regarding the topic "Owners' and veterinarians' perception" (topic 3), have surfaced through the years. Records related to this topic are usually based on questionnaires and surveys that are given to pet owners and veterinarians to express their opinion on different matters of welfare such as during vet visits (61–63) and the management of pet home (64). This topic included many records ($n = 228$) in line with the use of questionnaires in veterinary and animal sciences. In the case of pet welfare, the use of online surveys and telephone and face-to-face interviews has been useful to understand in depth the perception of owners and vets on several welfare-related topics, including cosmetic surgery (65) and pet management, namely training for car drives (66, 67) and veterinary examinations. For instance, Park et al. (68) clarified the relationship between American dog owner characteristics and willingness to seek veterinary care, while in their review La Vallee

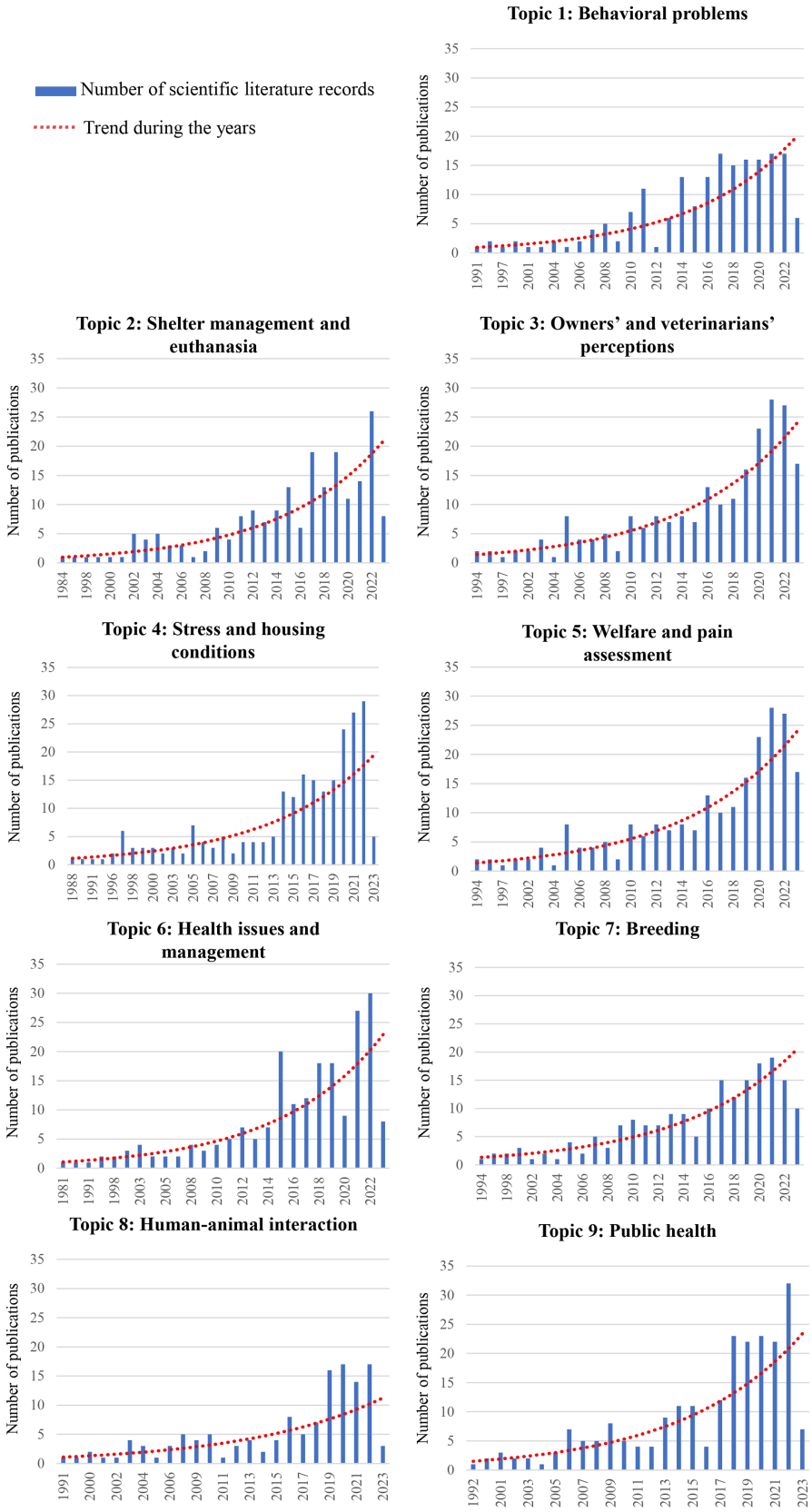

FIGURE 8
Graphs of the 1,775 records' distribution over the years 1980–2023 for each topic. The blue lines indicate the number of scientific literature records per year and the dashed lines indicate the trendline for the whole timespan.

et al. (69) listed cost, accessibility, veterinarian-client communication barriers, and lack of client education as the most common barriers to veterinary care. Education of owners was also pointed out by Park et al. as a crucial point to improve pet welfare (68).

The research topics named "Behavioral problems" (topic 1) and "Human-animal interaction" (topic 8) had a rise in the number of studies after 2010. This could be because behavioral medicine is a quite novel branch in veterinary medicine, so behavioral problems have started to be diagnosed accurately only in the last decades. A meta-analysis comparing the reasons for dogs being surrendered to shelters revealed that behavioral problems were the most common reason in eight out of nine studies. The reported frequency of relinquishment due to behavioral problems in a retrospective study by Jensen et al. ranged from 11 to 34% (70). The word "training" was associated with this topic and that is because training is a method of managing behavioral problems. A study conducted by Salman et al. (71), showed that owners of dogs facing behavioral problems are more inclined to seek training as a potential solution to address these issues and often have higher expectations for dogs that are perceived as "trained" compared to those considered "untrained." Pet ownership and the emotional attachment to pets are influential factors that can directly contribute to improved health and emotional well-being of pet owners (72). "Human-animal interaction" is a growing topic that centers around the bond and connection between pet owners and their animals. Studies conducted among American adults (73) and Israeli adults (74) reveal that an increasing number of pet owners consider their dogs or cats as integral members of their family. Words such as "impact" and "social" reflect the influence of pet ownership. Indeed, evidence from epidemiological and psychological studies suggests that pet ownership is associated with several positive health benefits for pet owners (75) and promotes social interaction (76, 77).

"Breeding" (topic 7) and "Welfare and pain assessment" (topic 5) were also studied mostly in the last decade. This could be firstly because selective breeding has become a welfare concern only recently, leading to the ban of the breeding of specific brachycephalic breeds in North European countries. Secondly, because commercial breeding of cats and dogs has been under-regulated worldwide, with a very limited number of studies focusing on the welfare of cats and dogs used for breeding (30). The topic "Welfare and pain assessment" did not contain many records (only 166); however, a higher number of studies was recently published probably due to the fact that the need to objectively measure welfare and pain is quite recent in the literature. A significant amount of research has focused on animal welfare problems, including the development of assessment methods for different environments (53). A scoping review published in 2021 found only a few studies focusing on the welfare and quality of life assessment of shelter dogs and all of them were published not earlier than 2010 (78). The first pain scale based on facial expression in cats was also published only in 2019 (79). The need for more studies aiming at identifying thresholds and aggregation methods to carry out risk analysis in animal welfare was pointed out by the European Food Safety Agency-EFSA (30).

The statistical approach was useful also to highlight other fields not investigated so far in relation to welfare. It is worth noting, indeed, the lack of knowledge and research found to exist about the principle of "Good Feeding." This could be due to the fact the word "welfare" was not used in the studies focusing on pet nutrition, as nutritionists do not see these two fields of research as interlinked. Another explication could be the fact that pets have usually access to food, so they rarely suffer from prolonged hunger, and consequently, this has not been seen as a welfare issue. On this matter, it is instead important to increase the research on appropriate feeding, which does not only mean offering a diet that covers the energetic requirement but also which meets the behavioral needs of our companion animals, also preventing obesity. Lund et al. (80) found out that 35% of household cats in the United States are obese and according to the Vet Charity for Pets in Need (PDSA) reports, veterinarians in the United Kingdom have witnessed an increase in pet obesity the recent years (81). More research on this topic should be consequently recommended. It should also be noted that there was a lack of research on the welfare of dogs and cats kept as laboratory animals. Dogs and cats have long been utilized in biomedical research due to their anatomical, physiological, and disease-response similarities to humans (82). The welfare considerations for laboratory dogs and cats are fundamentally the same as those for pets, even though the underlying motivations for these decisions may vary. Concerns for animal welfare and advancements in veterinary practices are collectively driving the exploration of alternative approaches to enhance the welfare of animals in laboratory settings among farm and pet animals (83). Furthermore, a less-explored topic was the positive welfare approach. The traditional approach to animal welfare was that negative physical or mental experiences should be minimized, while advances in the understanding of animals with the evolution in societal views have led to the gradual inclusion of positive experiences into definitions of "animal happiness" (84). So, it is increasingly acknowledged that considering only the negative aspects of animal welfare is not enough and by disregarding the positive aspects, there is less recognition of important factors related to animal behavior, physiology, and the considerations that owners naturally consider. These considerations include the animals' preferences, and their overall quality of life (85).

Our findings need to be interpreted with caution as several limitations should be considered as typical of the statistical method applied. Firstly, the search is strictly related to the keywords, so, although the search strings for entry into the Scopus® search were discussed in detail within the research team, some synonyms (e.g., "feline," "canine," and "well-being") have not been included and consequently our results may be underestimating the relative literature. Similarly, the search was limited to a single database, namely Scopus®, and thus some records published in journals not included in it may have been missed. Moreover, certain predetermined parameters were set before starting the research, including the restriction to English-only language records. Additionally, the adopted screening criteria may have resulted in a partial reduction in the number of records that were thoroughly analyzed. It is important to note that in this method of analysis, the 1,775 records were not read in their entirety but only the titles and abstracts were taken into consideration. Nevertheless, it is important to emphasize that the technique used might not have revealed other topics that could be more recent or of lesser scientific interest. Finally, only text mining and topic analysis were performed, but other statistical analysis, such as text mining on multi-word phrases and cluster analysis, have not been performed.

Notwithstanding those limitations, this review extensively examined the literature concerning the welfare of cats and dogs and it

successfully identified the research areas that have been extensively studied as well as the subjects that require further scientific evidence. Consequently, this review contributes as a valuable resource for future researchers, providing a foundation for further research in less-explored areas.

5. Conclusion

This review analyzed the literature related to the welfare of dogs and cats using machine learning methods. It found that dog and cat welfare is a growing field and that at least 9 different topics related to pet welfare could be identified as areas of research that have been studied to a greater or lesser extent over the past 40 years. There is a lack of research in areas such as optimal feeding practices, positive welfare, and the welfare of cats and dogs used as laboratory animals. Given that future legislation to protect the welfare of cats and dogs will need to be based on research, further studies are recommended to enhance our understanding of the welfare needs of companion animals and how to ensure positive welfare for them. More studies and reviews addressing companion animal welfare topics are therefore recommended.

Author contributions

CA: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. BB: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Writing – original draft. MZ: Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Writing – review & editing. MF: Conceptualization, Funding acquisition, Methodology, Resources, Writing – review & editing. NM: Investigation, Writing – review & editing. AlP: Investigation, Writing – original draft. AmP: Writing – review & editing. BP: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was funded by the Framework partnership agreement GP/EFSA/ALPHA/2021/10, Lot 1, signed between EFSA and the authors (BP, MF, and MZ).

Acknowledgments

The paper was born within the framework of the programmatic initiatives of the ASPA Commission for Animal Behavior and Welfare, and the authors are thankful to Barbara Cortiero who taught these machine-learning techniques to the members of the Commission. The authors would also like to thank Elizaveta Golunova for her help in the search and data entry.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

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frontiers | Frontiers in Veterinary Science

TYPE Review
PUBLISHED 02 November 2023
DOI 10.3389/fvets.2023.1284869

OPEN ACCESS

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RECEIVED 29 August 2023
ACCEPTED 12 October 2023
PUBLISHED 02 November 2023

CITATION
Littlewood KE, Heslop MV and Cobb ML (2023) The agency domain and behavioral interactions: assessing positive animal welfare using the Five Domains Model.
Front. Vet. Sci. 10:1284869.
doi: 10.3389/fvets.2023.1284869

The agency domain and behavioral interactions: assessing positive animal welfare using the Five Domains Model

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Animal welfare denotes how an animal experiences their life. It represents the overall mental experiences of an animal and is a subjective concept that cannot be directly measured. Instead, welfare indicators are used to cautiously infer mental experiences from resource provisions, management factors, and animal-based measures. The Five Domains Model is a holistic and structured framework for collating these indicators and assessing animal welfare. Contemporary approaches to animal welfare management consider how animals can be given opportunities to have positive experiences. However, the uncertainty surrounding positive mental experiences that can be inferred has resulted in risk-averse animal welfare scientists returning to the relative safety of positivism. This has meant that aspects of positive welfare are often referred to as animal 'wants'. Agency is a concept that straddles the positivist-affective divide and represents a way forward for discussions about positive welfare. Agency is the capacity of individual animals to engage in voluntary, self-generated, and goal-directed behavior that they are motivated to perform. Discrete positive emotions are cautiously inferred from these agentic experiences based on available knowledge about the animal's motivation for engaging in the behavior. Competence-building agency can be used to evaluate the potential for positive welfare and is represented by the Behavioral Interactions domain of the Five Domains Model. In 2020, The Model was updated to, amongst other things, include consideration of human-animal interactions. The most important aspect of this update was the renaming of Domain 4 from "Behavior" to "Behavioral Interactions" and the additional detail added to allow this domain's purpose to be clearly understood to represent an animal's opportunities to exercise agency. We illustrate how the Behavioral Interactions domain of The Model can be used to assess animals' competence-building agency and positive welfare. In this article, we use the examples of sugar gliders housed in captivity and greyhounds that race to illustrate how the agentic qualities of choice, control, and challenge can be used to assess opportunities for animals to exercise agency and experience positive affective engagement.

KEYWORDS

agency, animal welfare, positive animal welfare, positive affective engagement, quality of life, good life, happiness, animal wellbeing

1. Introduction

Animal welfare is both an academic discipline and a property of sentient animals. Animal welfare has been described as multi-disciplinary (1); however, it is increasingly becoming a trans-disciplinary field as it draws from and interacts across disciplines such as animal welfare science (including neurophysiology, applied ethology, and animal science), animal ethics (including philosophy and bioethics), psychology (including beliefs and attitudes, social psychology, and human behavior change), education, communication, animal law, and policy.

As a property of sentient animals, animal welfare represents how an animal experiences their life. Animal welfare, in this context, is a state within an animal. There are myriad definitions used to express this sentiment. However, the most consistently important concept for an animal is a focus on its subjective mental experiences. These mental experiences can vary from positive (e.g., pleasure from a comfortable environment, companionship from conspecifics, feeling well-fed) to negative (e.g., discomfort due to thermal extremes, loneliness, and a feeling of thirst) and can change over time (2). Added to this understanding that mental experiences matter to an animal, those mental experiences hold ethical relevance to the people who interact with animals (3, 4). Mental experiences underpin many animal laws [e.g., (5, 6)] that focus on preventing unnecessary or unreasonable suffering (i.e., suffering is a catch-all term for a range of negative mental experiences). A methodology for assessing animal welfare that focuses on an animal's mental experience is increasingly considered best practice in contemporary animal welfare science (1). This way of assessing animal welfare also creates unity within the discipline by aligning with the experiential focus of other facets (i.e., ethics, policy, and laws). In this article, an animal's *welfare* refers to its overall mental (affective) experiences.

This way of understanding animal welfare can pose challenges when it comes to welfare assessment. Most importantly, mental experiences are felt by the individual animal – they are subjective – and cannot be directly measured. This can be difficult for those accustomed to measuring other quantifiable features of animals, such as reproductive success, body weight, or heart-rate variability. Scientists can find that stepping over Dawkins' 'bridge' from the measurable and observable to the inferential and deducible makes them confront long-held beliefs and values (e.g., positivism) inherent in science [e.g., (2–4)]. However, affective neuroscience and studies in applied ethology allow us to make cautious inferences about relationships between measurable features of animals and their subjective mental experiences (7–13).

Animal welfare, conceptualized as the mental experiences of animals, can also make inferences about positive welfare challenging (14). Given that "good" animal welfare represents an overall positive welfare state, or a good life, for an animal (i.e., when opportunities for animals to have predominantly positive mental experiences are provided), how can positive welfare be assessed in a scientifically robust manner? We propose that the way forward is to consider animal agency.

Agency represents the new frontier in animal welfare assurance. While traditional animal welfare management has focused almost exclusively on minimizing animal welfare compromise, or "suffering," contemporary approaches consider how animals can be given opportunities to experience positive welfare (3, 14–17). For example, standards of care have historically focused on security and physical health aspects of animal housing environments. Guidelines for dairy cattle specify, "Cattle without shelter need to put more energy into normal functioning and less into production" (18). Whereas modern standards now include additional consideration for the positive mental experience of animals, with provisions relating to bedding, cleaning, lighting, temperature, noise, ventilation, and humidity [e.g., (19)]. This is to ensure that animals do not only avoid discomforts that may be harmful but will be comfortable. More recently, positive animal welfare has been characterized by four features: positive emotions; positive affective engagement; quality of life; and happiness (14). We argue that each of these features can be linked to animal agency. More specifically, these features are more likely to occur when animals engage with opportunities to exercise agency.

Agency is the capacity of animals to engage in voluntary, self-generated, and goal-directed behavior that they are motivated to perform (20, 21). These behaviors can be motivated by positive affective consequences (22, 23). The collective term for these positive subjective mental experiences (or affects) resulting from reward-based motivations is "positive affective engagement" (23–25). This term reflects the engagement, or "flow," inherent in these experiences (26). Animals are pleasantly occupied [e.g., a detection dog engaged in a scenting task (26, 27)] to such an extent that they can become oblivious to other sensations or mental experiences – provided they are not significantly negative (23–26). Discrete positive emotions, or affective states, are cautiously inferred from these agentic experiences based on available knowledge about the animal's motivation for engaging in the behavior. Such motivations can be encoded at the species level and passed to the individual animal via their genome (phylogenetic) or occur at the individual animal level because of environmental interactions within the individual's lifetime (ontogenetic). The exact nature of these drivers and their impact on affective experiences are, as yet, poorly understood.

For this reason, positive welfare, or more precisely, the uncertainty surrounding mental (affective) experiences that can be inferred, has resulted in risk-averse animal welfare scientists returning to the relative safety of positivism. This has meant that aspects of positive welfare are often referred to as animal "wants" – and "needs" are the basic provisions that precede these "wants" (28–32). Framing animal welfare as "needs" and "wants" risks reducing human responsibility towards animals to solely neutralizing negative experiences ("needs"), while positive experiences ("wants") could be perceived as an optional luxury (33, 34). Agency is a concept that straddles the positivist-affective divide and represents a way forward for productive discussions about positive animal welfare and to help advance the welfare of animals under human care.

This article aims to articulate how agency can be used to assess animal welfare and the relationship between an animal's welfare and their ability to exercise agency. A secondary objective is to illustrate how the Behavioral Interactions domain (Domain 4) of the Five Domains Model represents this expression of agency.

2. The Five Domains Model and animal welfare assessment

When understood in affective state terms (i.e., a focus on mental experiences), animal welfare should be assessed in such terms (1). The

Five Domains Model is a framework for assessing animal welfare that focuses on subjective mental experiences that matter to the animal (35). Other animal welfare assessment frameworks exist. For example, Welfare Quality focuses on four areas: good feeding, good housing, good health, and appropriate behavior (36). However, none focus on the mental experiences of animals to the same extent as the Five Domains Model (35).

The structure of the Five Domains Model is illustrated in Figure 1. The first four domains represent inputs to the animal that are processed by their species-specific physiology and behavioral biology resulting in physical/functional states (Domains 1 to 3) or representing an animal's externally perceived situation (Domain 4) (35).

Domain 1 (Nutrition) and Domain 3 (Health) are the physical/functional states of the animal (e.g., nutritional or hydration status and physical health issues such as illness and physical dysfunction) that are the states most familiar to veterinary and animal scientists (37). Domain 2 (Physical Environment) focuses on conditions available to the animal (e.g., space allowance, air quality, bedding). Domain 4 (Behavioral Interactions) represents the animal's ability to exercise agency in their interactions with the environment, other animals, and humans (35).

The Model is a framework and focusing device for animal welfare assessment that needs to be operationalized for the specific context and animal The Model is used to assess (38). Valid welfare indicators need to be established for each of the states/conditions/agency initiatives in Domains 1 to 4. The second part of this two-step process requires that these welfare indicators be validated for the specific mental experience they can infer in Domain 5, Mental State (39).

Domain 5 (Mental State) represents the animal's overall welfare, or lived experience, in affective terms (35). This domain is not assessed separately, but rather it reminds users to draw affective inferences from states/conditions/agency initiatives identified in Domains 1 to 4. In this way, The Model takes an affective state approach to animal welfare assessment (35). Welfare impacts identified in Domains 1 to 4 must have corresponding mental experiences (inferred in Domain 5) that matter to the animal to impact their welfare (35).

Evidence from multiple disciplines (e.g., affective neuroscience, physiology, ethology, psychology) informs The Model's use and subsequent updates. In 2020, The Model was updated to, amongst other things, include consideration of human-animal interactions (35). The most important aspect of this update was the renaming of Domain 4 from "Behavior" to "Behavioral Interactions" and the additional detail added to this domain to allow its purpose to be more clearly understood. This domain had been understood by its authors as "The Agency Domain" for several years preceding this update. However, 2020 marked the year where there was a recognized need for Domain 4 to be renamed to link it more explicitly to an animal's ability to exercise agency (35). It was envisaged that this update would help readers better understand Domain 4 and the important role of animal agency in animal welfare assessment (15).

3. Behavioral interactions and domain alignment

Renaming Domain 4 of The Model to "Behavioral Interactions" (35) in 2020 was necessary to align it with the "input" focus of Domains 1 to 3. Domain 1, Nutrition, focuses on nutritional inputs (e.g., food and water provision) that may impact the animal's nutritional status in functional terms. Domain 2, Physical Environment, inputs are externally available conditions in the physical environment (e.g., ambient temperature, air quality). Domain 3, Health, is used for factors contributing to vitality, disease, injury, or other functional or physiological conditions contributing to an animal's physical health and fitness (e.g., parasite control, vaccination). Overall, Domains 1 to 3 focus users on various survival-related inputs and provide a structured approach to inferring how these inputs, and their effects on physical/functional states or available conditions, impact overall welfare (mental experiences) in Domain 5, Mental State (35).

Before the 2020 update, Domain 4 was called "Behavior" and was routinely used to describe an animal's outward behavioral expression. However, behavior is an indicator of welfare. Behaviors can be used across all four domains (e.g., shade-seeking behavior may be used in Domain 2 to evaluate the suitability of the Physical Environment an animal is kept within). The updated term 'Behavioral Interactions'

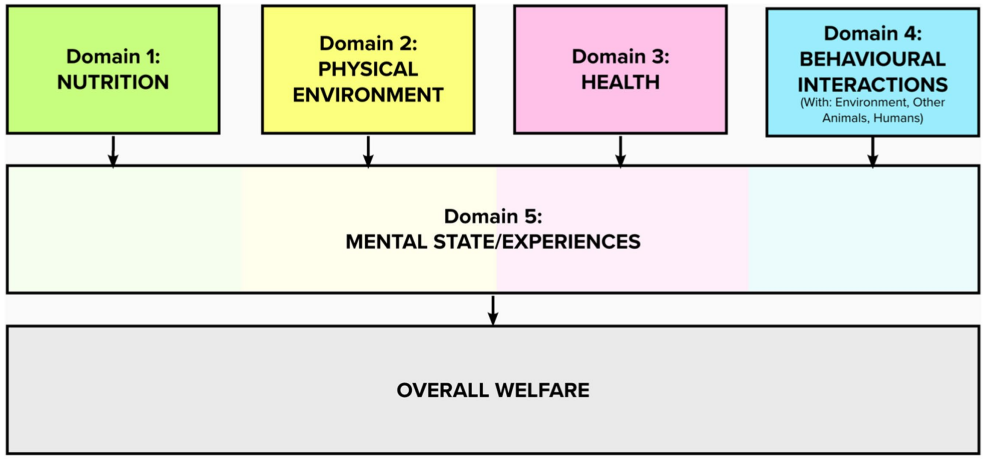

FIGURE 1
The 2020 Five Domains Model of animal welfare.

focuses on inputs to the animal that constrain or provide opportunities for animals to exercise agency (40). Three subcategories were included to encourage users to consider opportunities for animals to exercise agency during interactions with: (A) the environment; (B) other animals; and (C) humans (35).

Domain 2 was also renamed in 2020 from "Environment" to "Physical Environment" to clarify and help distinguish it from Domain 4 (35). Domain 2 focuses on provisions and aspects of the environment that contribute to an animal's physical comfort. In contrast, Domain 4 (specifically the subcategory of 'interactions with the environment') focuses on parts of the environment an animal interacts with and the ways an animal interacts with these features (35).

3.1. Environmental enrichment

Behavioral Interactions (Domain 4) is where environmental enrichment is considered (35, 37) within the Five Domains Model. Environmental enrichment refers to structures and stimuli that promote species-specific behavior that is important and beneficial from the perspective of an individual (41). This means that environmental enrichment broadly corresponds to features that give animals opportunities to exercise agency. Different types of enrichment have been articulated: occupational, physical, sensory, nutritional, and social (42). However, environmental enrichment can be difficult to apply when aligned with the Five Domains Model and the affective state orientation to animal welfare. Firstly, enrichment types are not all 'environmental' in their application. Occupational enrichment can result from interactions animals have with other animals, humans, or even smart technologies (43). Social enrichment, by definition, occurs during interactions with other animals and humans. When using Domain 4, it may be more beneficial to align enrichment types with the different sub-categories of this domain: Environment, Other Animals, and Humans (Table 1).

Operationalizing the term "enrichment" can be challenging. Environmental enrichment originated in laboratory animal welfare as a compensatory device but has become an increasingly essential tool for providing animals in managed captive settings with opportunities for positive welfare (44–46). Environmental enrichment is now used across zoos and aquaria (44, 47) and is increasingly reported in other settings (e.g., farm animals with enrichment opportunities such as brushes and showers). Environmental enrichment has undoubtedly led to improved animal welfare (48). However, it may have reached a point where the term 'enrichment' no longer aligns with contemporary animal welfare science thinking.

Enrichment implies an optional improvement that can be used in any setting to improve animal welfare. However, animals experiencing significantly negative mental experiences, for example, those raised in isolated and barren environments that do not provide agentic opportunity for social and exploratory behaviors, may be unable to respond to environmental enrichment features [e.g., captive bottlenose dolphins isolated in quarantine did not engage with enrichment toys (49)] (15). Enrichment cannot be treated as a panacea for all issues of welfare compromise or to legitimize housing animals in unsuitable conditions. Instead, there is a need to assess an animal's welfare systematically and holistically across multiple domains to understand the best way(s) to optimize their welfare. For this reason, a more appropriate way forward may be to rephrase this concept as 'environmental optimisation' or 'environmental challenge' (21). Optimisation is more nuanced and implies a greater understanding of the underlying animal welfare compromise and the targeted strategies that should be developed to ameliorate it and bring about welfare improvement.

As a term, *environmental enrichment* has become synonymous with welfare improvement and is entrenched in many people's minds. Thus, reframing its meaning may be a more effective way forward rather than changing the term. Fernandez argues that environmental enrichment was never meant solely to provide animals with *objects. Instead, it* refers to stimuli and/or events that result in animals having opportunities for enriched *quality of interactions* with their environment, other animals, and humans (50). Positive reinforcement training can modify these interactions and function as an enrichment [e.g., training promoted social interactions by moderating chimpanzee aggression during feeding (51)] (50). This framing aligns with the concept of agency and the interaction subcategories of Domain 4. The structured framework of The Model can be used to identify specific enriching interactions and then direct carefully considered and targeted interventions (35).

TABLE 1 Types of environmental enrichment (42) and their alignment with sub-categories of Domain 4.

Enrichment aligned as behavioral interactions with...		
The Environment	Other animals	Humans
Occupational, e.g., cognitive (puzzles, activities), exercise (mechanical, run) Physical, e.g., enclosure (size, complexity), accessories (items) Sensory, e.g., visual (windows), auditory (vocalizations), olfactory Nutritional, e.g., delivery (frequency, schedule), type (novel, variety)	Occupational, e.g., cognitive (group activities), exercise Social, e.g., contact (conspecific/non-conspecific), non-contact (visual, auditory, olfactory)	Occupational, e.g., psychological (training activities), exercise Social, e.g., contact, non-contact (visual, auditory, olfactory)

4. The agency domain and animal welfare

Agency is the capacity of animals to engage in voluntary, self-generated, and goal-directed behavior that they are motivated to perform (20, 21). These behaviors can be motivated by positive affective consequences, i.e., those that result in positive affective engagement, or by negative affective consequences (e.g., avoiding predation or other situations perceived as a threat) (7, 8, 52). Špinka describes three ways to understand the welfare benefits of animals having the capacity for agency: adaptive functioning, affective functioning, and awareness/selfhood (52). From the adaptive point of view, goal-directed behavior confers a survival advantage to animals. An animal that approaches interactions (with its environment, other animals, and/or humans) reactively or reflexively [e.g., the starfish has a righting reflex in response to inversion (53)] is less likely to survive in complex environments than one that has the cognitive capacity to

be proactive (flexible) in its interactions (52). For example, wild deer fawns with mothers who proactively hid their young were more likely to survive in open habitats than reactive-mothered fawns (54). Conversely, expressing agency may be less critical to animals in simple environments with relatively stable interactions.

The affective functioning viewpoint focuses on evidence from affective neuroscience and an appreciation of the neurobiological mechanisms underpinning mental (affective) experiences (52). Fundamental to this viewpoint is the understanding that mental experiences are motivational forces (or drivers) for the complex behaviors animals perform (16). In other words, mental experiences are proximate causes of complex, but not reflexive, behavior (55, 56). More complex agentic capacities require more diverse underlying mental experiences. Animals operating competently within complex environments might be expected to possess a greater range of mental qualities because of a need to exercise greater agency.

Differing levels of awareness is another way of conceptualizing the welfare benefits of animal agency. In this conceptualisation, consciousness or self-awareness accumulates at different levels (52). The most basic level of awareness includes a sense of 'core self' that allows individuals to identify sensations and behaviors as their own in the present moment (7). The next awareness level relates to competence-building (57). At this level, the animal has the capacity for cognitive processes such as learning and memory, enabling them to accumulate skills and knowledge from previous experiences. In other words, animals can build competence towards a species-specific level of awareness when given opportunities to exercise agency. Long-term goals and aspirations are features of the highest awareness level and result from decision-making based on introspection (52). An animal's umwelt, or unique perceptual world, is dictated by its awareness level (58). Therefore, a higher level of awareness gives a broader scope for umwelt.

Overall, Špinka identifies three ways agency relates to positive animal welfare (52). First, agency can be competence-building, and animals given opportunities to exercise agency are more likely to develop the skills (e.g., physical strength, social cohesion, mental resilience) necessary to overcome future agentic challenges. In other words, animals learn when they can exercise agency. Play in young animals is an example of this agentic learning process (59). Second, animals with opportunities to exercise agency can also experience positive affective engagement (i.e., a range of positive mental experiences), for example, pleasure, affectionate sociability, and care (15, 38). Finally, it is proposed that competence-building is welfare-enhancing as it supports the development of species-specific higher levels of awareness and allows an animal's full interactive potential, and umwelt, to be met (52). At a higher level, this could result in animals, with the phylogenetic capacity, attributing meaning to their lives – a feature used to classify human happiness (52, 60), refer Figure 2.

4.1. Competence

A detailed exploration of agentic qualities such as competency, choice, control, challenge, and umwelt can further articulate agency. Competence results when an animal has the tools and strategies to deal with novel and ongoing challenges (31, 52, 61, 62). In other words, competency is the outcome of animals' opportunities to exercise agency during their lifetime. The strategies for behavioral interactions (with the environment, other animals, and humans) have developed because of these opportunities, i.e., competence is agency-driven ontogenetic development (31, 57). Competence can enable future agency and be an outcome of exercising agency. The characteristics and skills developed during opportunities to exercise agency can enable animals to act with self-determination and increase their opportunities for agency (57).

Enhanced functional (e.g., physical conditioning) and cognitive (i.e., learned) capacities contribute to competence. Lack of space or incentive to exercise vigorously can result in poor physical conditioning, often exacerbated by uniform and limited opportunities for interactions with the environment (63). An individual animal unable to satisfy its genetic (phylogenetic) and developmental

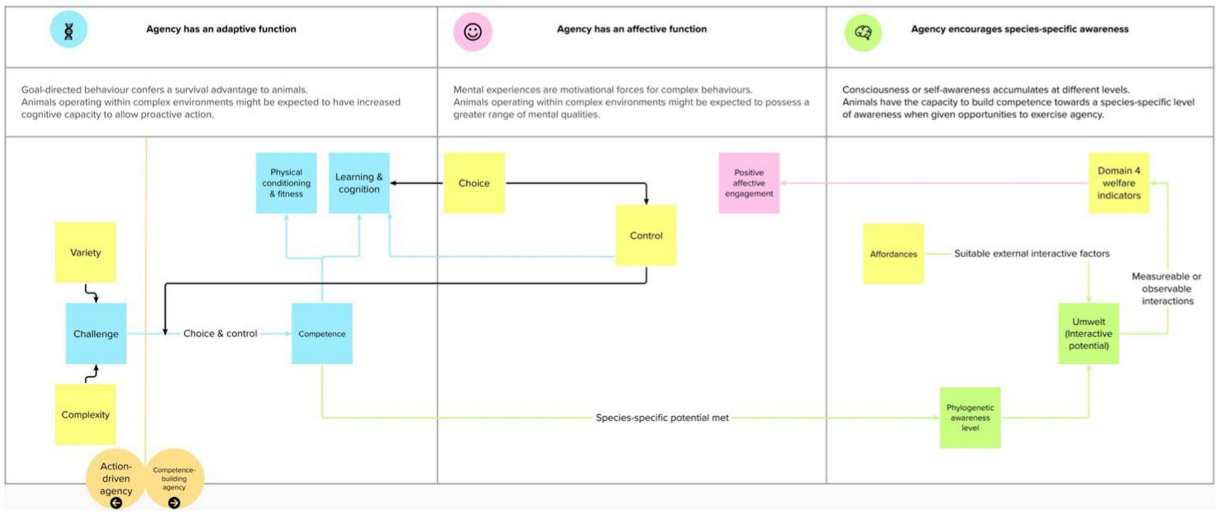

FIGURE 2
The three ways Špinka (52) relates agency to positive welfare and their relationship to other concepts used in animal welfare science.

(ontogenetic) competence potential may experience a form of learned helplessness (63). Conversely, an animal in a barren environment may have developed less competence and a reduced threshold to perceive novelty (64). This could lead to arguments against providing animals in captivity with environmental enrichment opportunities. However, individuals with low competence living in captive environments should still be provided opportunities to exercise agency.

Suppose environmental enrichment provides opportunities for animals to exercise agency and develop competency. For example, they may become more challenging animals to contain in a zoo setting. In that case, a potential solution is to restrict opportunities for agency (e.g., by withholding enrichment) to limit escalating enrichment requirements. However, agency is required for animals to develop optimal physical functioning (e.g., via play) as well as mental capacities (26, 34, 65). Agency is also self-fulfilling and provides animals with opportunities to experience positive affective engagement in novel ways or ways that cannot otherwise be provided (15, 38). And even with restricted opportunities to exercise agency, and thus blunted competence, many animals retain the pre-programmed genetic potential (i.e., motivation) for agency due to phylogenetic developmental events within their species (31, 65). Impeding agency is in and of itself a welfare compromise, independent of how lowered competency may influence the perception of further welfare-compromising conditions. Ethically, if we are aware of these agentic requirements of animals (i.e., a valid evidence base exists), people are morally obligated to provide them. We anticipate this obligation will feature increasingly in the safeguarding, welfare assurance, standards of care, regulations, and animal management legislation in the coming years.

4.2. Choice

Choosing between two or more options allows animals to exercise agency (52, 57, 61, 63). Agentic "freedom of choice" roughly aligns with one of the Five Freedoms; "freedom to express normal behavior" (66, 67). However, providing for choice requires animals to have uninhibited options that align with their species-specific motivations (68). This requires detailed knowledge of what is normal for a species to do (i.e., knowledge of their behavioral biology). The domestication process has changed the behavioral biology of some animals to such an extent that 'normal' may cease to exist at the species level (3). Comparisons to wild populations cannot always be relied upon as many domesticated species no longer resemble their wild ancestors (3). Also, there is still much to learn about the behavioral biology of a range of taxa (69, 70). Added to this, there can be marked differences in the preferences of individual animals (52, 63).

Consequently, even at the species level, "normal" behavior represents a generalization that may not be informative when assessing the welfare of an individual animal. Overall, these considerations make it difficult to predict the behaviors an animal may want the "freedom to" perform. Affording animals agentic choice offers more versatile options for positive welfare, such as using technologies (40), than providing animals with contexts to perform specific "normal" behaviors – when they are known. Additionally, animals may prefer fewer choices than those offered to them or may prefer to interact with a choice not offered in managed settings.

Active environmental enrichment represents an example of agentic choice. Active enrichment is something an animal engages with directly through agentic choice being provided (e.g., food hidden in a tree to be detected and secured). In contrast, passive enrichment is provided to the animal without agentic choice (e.g., music is played) and may be not be perceived as rewarding by the animal (65, 71).

4.3. Control

Choice and control are interrelated aspects of exercising agency. Control is realized when an animal can *consistently* and *predictably* make choices and obtain the outcomes they are motivated to achieve (61, 65, 72). When animals can actively decide when and how to interact with the environment, other animals, and humans, they have an element of control over their choices (52, 57, 65, 72). Inaction is as essential as action; an animal choosing not to interact (e.g., with a toy offered to it) exerts control over its actions and therefore exercises agency (57, 65). Perception of control, whether exercised or not, influences cognition and behavior in animals responding to challenging situations (62, 65).

Perceived control forms the basis of cooperative care protocols and animal consent (73). Cooperative care involves training animals to make informed choices (i.e., consent) about their management (74). These training protocols should allow animals to consent and withdraw their consent at any time. Chin rest is an example of a common consent behavior used in dogs (74). Informed choice involves some level of predictability (i.e., control) and allows animals to exercise agency by controlling what happens to them (74). For example, automated technology can enable dairy cows to control their engagement with mechanical grooming brushes (75). When an animal can exert control, they may be more likely to engage in challenging interactions and develop competence (65).

4.4. Challenge

Various complex interactions can challenge animals and encourage the development of problem-solving abilities that confer competence (21, 46, 64, 65). Novelty increases the variety of interactions an animal may have. An animal can be provided with difficulty by making situations or tasks challenging to analyze, understand, or solve such that learning occurs (57, 61). Physical challenges can also offer advantages to animals by improving physical conditioning and fitness (64). Care must be taken to ensure challenges are not too far beyond the competency level of the individual animal as this can have negative affective consequences, e.g., result in frustration or anxiety (64). Suppose these challenges do not far exceed an animal's current competency level (i.e., they are surmountable). In that case, they offer an opportunity for the animal to exercise agency and experience positive affective engagement (15, 38). Examples of so-called 'environmental enrichment' challenges, and their alignment within Behavioral Interactions, Domain 4, are presented in Table 1.

4.5. Umwelt and affordances

How an individual animal feels about its competence also matters for its welfare. An animal's umwelt represents its unique perceptual and effector world, i.e., an animal's inner world (58). A higher level of awareness gives a broader scope for umwelt (52). The concept of

umwelt has the added advantage of considering the differences in sensory worlds between animal taxa (58). Umwelt goes beyond sense organ physiology and considers how an animal responds to their situation and how these responses modify their perceptions of self and subsequent interactions with the environment, other animals, and humans (58).

In their discussion of animal communication, Parton and Marler (58) liken umwelt to Gibson's theory of affordances, which describes the relationships between animals and their environments (76). Affordances of the environment are what it offers the animal, good or bad (76). An affordance is measured relative to the animal and is unique to that animal rather than measured in abstract physical properties (e.g., load-bearing force). Because affordance is interpreted relative to the perceiver (76, 77), an animal's unique perceptual world (umwelt) will impact its perceived affordances (58). Gibson (76) suggests that an animal's ecological niche is a set of affordances. A niche is how an animal lives and their role, rather than the habitat or where they live (76). An animal's perceived affordances may determine their ability to develop competence and, in turn, impact how they can exercise agency.

Each of the terms interact to provide an overview of how animals might be provided opportunities to exercise agency to engage in voluntary, self-generated, and goal-directed behaviors that they are motivated to perform (20, 21), as depicted in Figure 2.

5. The agency domain in action: assessing animal welfare

Špinka's four tiers of agency can help further articulate the role of animal competency when using the Five Domains Model to assess animal welfare. These tiers are passive/reactive agency, action-driven agency, competence-building agency, and aspirational agency (52). The tiers are distinguished by the type of behavioral interaction an animal has, which relates to the dominant brain structure(s) and awareness level(s) operating.

Passive/reactive agency is characterized by passive or reflexive reactionary behaviors resulting from external stimuli. Most are driven by homeostatic and sensory affective states involving the brainstem or corresponding neural substrate in non-mammalian animals (52). The resultant drives are probably subconscious and unlikely to play a role in animal welfare and assessment. For example, moon jellyfish (*Aurelia* sp.) dive in response to turbulence (78).

Action-driven agency involves emotional action systems at the subcortical level (52). The resultant behaviors are mostly survival-related, aimed at procuring food, seeking shelter, and avoiding predation. This tier aligns most with Domains 1 to 3 (Nutrition, Physical Environment, and Health) and is not the 'interactive' agency considered in Domain 4 (Behavioral Interactions).

Competence-building agency involves active behavioral interactions to build skills and acquire information for later use. This tier involves learning-related emotions at the level of the basal ganglia or corresponding neural substrate in non-mammalian animals (52). Such activities are future-focused and, rather than achieving immediate outcomes, allow animals to enhance skills and gather information (i.e., develop competence) for future use. Examples include instrumental and social learning, exemplified by contrafreeloading whereby animals choose to work for food over obtaining freely available food (50, 78). Inspective and inquisitive exploration, communication, and some forms of play also fall within this tier (52). This tier most closely aligns with the operational intent of Domain 4 (Behavioral Interactions). In other words, competence-building agency is the construct being assessed when the Behavioral Interactions with the environment, other animals, and people in Domain 4 is used as part of a holistic welfare assessment protocol.

Aspirational agency is driven by an animal's neocortex and allows for complex interactive behaviors resulting from planning and goal setting. These often involve *affectively* guided planning and intentions to act (52). However, the evidence thus far suggests that this agency level is less prominent in non-human animals. Therefore, this level of agency is not currently considered within the Five Domains Model of animal welfare assessment but does encourage debate about how an animal's time perception and planning may be considered in future updates to The Model.

Given the traditional focus of animal welfare science on the biological functioning orientation and alleviating welfare compromise (3, 79), we have amassed substantial information that contributes towards our understanding of negative mental experiences aligned with Nutrition, Physical Environment, and Health, Domains 1 to 3. Behavioral Interactions, represented in Domain 4, and their aligned mental experiences have proven more challenging to study empirically. This most likely stems from the difficulty scientists face when attempting to develop paradigms to evaluate agency robustly. This is particularly true for mental experiences traditionally assigned a positive valence (25, 34). However, as mentioned, we should avoid returning to the relative safety of positivism, where any reference to mental experiences is side-stepped. Instead, these challenges encourage us to exercise extra caution when considering mental experiences aligned with Behavioral Interactions and the expression of agency (Domain 4). Moving forward, animal welfare assessment using Domain 4 could be performed by reflecting on an animal's ability to exercise various qualities of agency (see Section 4 of this paper) and aligning these to the experience of positive affective engagement (a catch-all term for positive mental experiences related to exercising competence-building agency) (15, 38). The terms 'pleasure' or 'happiness' could be used to reflect this when communicating with a lay audience.

5.1. Impediments to agency being exercised

Negative mental experiences inferred from impacts in Behavioral Interactions (Domain 4) result from impediments to an animal's ability to exercise competence-building agency. These negative experiences reflect the cognitive responses of animals to being kept in impoverished environments (e.g., a laboratory rat in experimental deprivation conditions), under firm behavioral restriction (e.g., a working guide dog that cannot actively explore by sniffing or interact with other people or animals it encounters), or confronted by threatening situations (e.g., a horse kept with resource guarding conspecifics). This helps explain why these negative experiences have been collectively termed 'situation-related negative affects'; they reflect the animal's perception of their external circumstances, i.e., their situation (15, 35).

Impoverishment is a feature of restricted opportunities to engage in interactive behaviors – with the environment, other animals, or humans. Examples of these restrictions include limited space, barren or invariant features in enclosures, and social animals with little or no access to the company of others (15, 80). The development of negative mental experiences in restricted circumstances is believed to result from thwarted genetically pre-programmed (phylogenetic) or learned (ontogenetic) motivations to engage in rewarding behaviors or behaviors that result in a reward (7, 8, 15, 21). Such adverse experiences inferred (in Mental Experiences, Domain 5) from restricted circumstances may initially include frustration and fear (e.g., short-term kennelling of dogs) and then give way to boredom, depression, helplessness, loneliness, and isolation (8, 20). These latter mental experiences may promote low activity and energy conservation where resources are limited (81, 82). In other words, these mental experiences may result from loss or lack of reward following unsuccessful attempts to engage in highly motivated behaviors, i.e., when competence-building agency has been impeded.

Interactions (with the environment, other animals, and humans) that are cognitively perceived as threatening are also aligned with Domain 4, consistent with the positive and negative inputs possible in Domains 1–3 (Nutrition, Physical Environment, Health). Examples of potentially threatening situations include possible or actual attack, separation from the security and protection of others of social significance, and overstimulation or being presented with challenges that an animal has not developed competence to manage or avoid (15). Negative experiences inferred (in Domain 5, Mental State) from threatening situations may include anxiety, fear, and panic (8, 15). These negative mental experiences align with Mendl et al.'s upper left quadrant, i.e., Q4 of the functional core affect model, resulting from a desire to avoid aversive situations (81, 82). They promote coordinated responses to the presence of threat or danger. Such experiences are unlikely to be competence-building if the circumstances impede an animal's ability to exercise agency through choice and control (e.g., victimization in a confined space).

5.2. Opportunities to exercise agency

Positive mental experiences inferred from Behavioral Interaction factors in Domain 4 are attributed to animals having opportunities to exercise agency and express more of their behavioral repertoire (15, 35). Correction of impacts in Nutrition, Physical Environment, and Health (Domains 1 to 3) that generate survival-related negative Mental Experiences (Domain 5) may enable the animal to refocus on engaging in rewarding behaviors. In other words, survival-related negative mental experiences at high intensities (i.e., compromised welfare) dominate the overall mental experiences of an animal, but when minimized, allow the animal to exercise agency and experience positive affective engagement (15, 38). This could be akin to an animal experiencing an overall feeling of physical safety when survival-related experiences aligned with Domains 1 to 3 are mitigated (83). Once physically safe, animals are more likely to engage in the rewarding Behavioral Interaction activities of Domain 4 (83).

Short-lived positive experiences may be generated from survival-related behaviors motivated by negative mental experiences (15). Water drinking behavior (Domain 1) initiated by the negative experience of thirst (Domain 5) may also result in transient positive experiences such as oral wetting and quenching pleasure (13). Such positive mental experiences may reduce or replace negative experiences but are unlikely to contribute to an overall positive welfare state long-term (15).

In contrast, some situation-related negative experiences may be replaced by positive ones when improvements are made to interactions (with the environment, other animals, and/or humans) that allow animals to engage in more rewarding behaviors (13, 17). For domestic species kept in human-dependent conditions, the negative experiences generated by such impeded interactions (i.e., impeded agency) often require intentional human intervention to correct. Again, providing opportunities to engage in rewarding behaviors is the basis of environmental enrichment strategies (44). Enrichment initiatives can serve to promote positive mental experiences (15, 38).

As mentioned in section 4.1, negative experiences (e.g., helplessness and isolation) can result from restricted circumstances (81, 82). Interventions to replace these negative experiences with positives (e.g., happy, excited) should focus on providing animals with opportunities to acquire rewarding experiences during their behavioral interactions (with the environment, other animals, and humans) (81, 82). Stimulus-rich and diverse or novel settings allow animals to engage in interactive behaviors, such as exploration and play, associated with positive experiences (15).

Potentially threatening situations can result in negative experiences such as anxiety and fear. These negative mental experiences likely result from a desire to avoid aversive situations (81, 82). However, when opportunities are provided for animals to build competence and exercise agency through choice and control, positive experiences (e.g., calm and relaxed) can replace these negative experiences (81, 82).

The precise valence and intensity of some individual mental experiences are still debated (e.g., boredom, helplessness) and likely vary depending on the individual's life experiences and the length of time they are experiencing these feelings. Further exploration is needed to develop our conceptual understanding of these mental experiences. However, strategies to support agency and positive affective engagement focus on providing animals with opportunities to exercise a maximal 'level of agency'.

6. Strategies to support agency and positive affective engagement

This section gives situational examples where animals can have competence-building agency and experience positive affective engagement. To illustrate this, we use two examples where opportunities for animals to exercise agency could be enhanced: sugar gliders kept as animal companions and greyhound dogs that race and are housed in kennels. Creating such opportunities for animals to exercise agency may require additional resources, such as space, equipment, or people's time.

Assessing the welfare of animals using the Five Domains Model requires a systematic approach using all five domains. When experiences aligned to Domains 1 to 3 (e.g., hunger, pain) are sufficiently negative, animals may be less motivated to engage with opportunities for competence-building agency (84). In other words, without an overall experience of physical safety and health, an animal is less likely to engage in activities they might have found rewarding

(83). However, given that this article focuses on the Behavioral Interactions (Domain 4), an abbreviated approach to identifying potential welfare impacts aligned with Domains 1 to 3 will be taken. This does not detract from the importance of a complete and systematic welfare assessment here; instead, it reflects a desire to focus specifically on elucidating the connections between Domain 4's behavioral interactions with the environment, other animals and people, and positive welfare.

6.1. Sugar gliders housed in captivity as companion animals

Sugar gliders (*Petaurus breviceps* and *P. notatus*) are small, nocturnal, arboreal marsupials, native to parts of Australia and Oceania (38). In the wild, they live in colonies of 10–15 individuals in open forests and have an omnivorous diet of gum, sap, and insects (38, 85). This species spends most of the night active in tree branches and can glide up to 50 meters between trees (38, 85). They are highly active and maintain a territory of up to 1 ha in the wild (38). Although keeping these wild animals is restricted or prohibited in many places, Sugar gliders are an example of a non-domesticated animal commonly kept as companion 'pocket pets' in several countries globally, including the United States (85). They have an average lifespan of 7 years in the wild but can live up to 15 years in captivity (38). They have a paedomorphic appeal that likely triggers an instinctual human attraction – often described as the "baby schema effect" (86). A set of infantile (or neotenous) features, perceived as cute, evoke a nurturing response from humans, i.e., their small size (12 to 15 cm in length), facial features that are large in comparison to their round head, and large, dark, wideset eyes (38, 86). When kept as companions, they often present with veterinary problems associated with inappropriate housing, activity and diet, e.g., obesity (85).

6.1.1. Domains 1 to 3

In captivity, welfare impacts aligned with Domains 1 to 3 are diverse. An inappropriate diet (Domain 1) is a common cause of sugar gliders presented to veterinary clinics (38). Many readily available diets show evidence of mineral and vitamin imbalances (38, 87). Diet-related conditions include malnutrition, obesity, osteodystrophy, and dental disease (38, 87). These will likely lead to mental experiences such as hunger, weakness, malaise, and pain. Sugar gliders tolerate temperatures between 18 and 32°C. Temperatures outside this range increase the risk of them experiencing various forms of discomfort and thermal extremes of chilling or overheating. Having sufficient space for spontaneous locomotion (Domain 2) and maintaining physical fitness (Domain 3) is also essential for positive welfare opportunities in Domain 4.

6.1.2. Domain 4

Examples of positive behavioral interactions aligned with Domain 4 are further sub-categorized into interactions with the environment, other animals, and humans (Table 2).

6.1.2.1. Interactions with the environment

In their natural habitat, sugar gliders are nocturnal and spend much of their awake time at night foraging for food, i.e., interacting with their environment. They use their long incisors to extract gum and strip bark from trees (38). When food is readily provided to captive sugar gliders, this not only increases their risk of developing obesity (Domain 1) but also reduces opportunities for them to perform feeding behaviors that build competence and would otherwise keep them occupied for extended periods (Domain 4) (38). Instead, materials that simulate foraging can be provided in captivity, e.g., holes drilled into non-toxic materials filled with food or other complex food toys (38). These are examples of occupational, physical, and nutritional enrichment strategies (Table 2) that allow sugar gliders to experience positive affective engagement.

Aviaries of sufficient size, particularly height, allow sugar gliders opportunities to glide between perches (38). These animals will also need branches- or rods arranged vertically and horizontally in their enclosure – to encourage scurrying, jumping, climbing, and gliding (38). Perches, swings, and ladders are valuable items in aviaries (38). Items resembling predators (e.g., clothing) should not be left where sugar gliders may perceive them as a threat, e.g., on top of cages, as this might limit their exploration and interaction with the full scope of available environment (38). For resting, a nest box should be provided in a suitably-sized aviary (85).

6.1.2.2. Interactions with other animals

Sugar gliders are vulnerable on the ground and prefer to remain elevated (85). Sugar gliders are often kept individually in small bird cages with a suspended pouch as a nest (85). Sugar gliders in the wild are territorial and can become aggressive if not introduced carefully (85). The social nature of sugar gliders means that most guidelines recommend housing them in groups of at least two in captivity (38). Sugar gliders prefer to sleep huddled together, so nests should be large enough to allow co-habitation (38). Cats and other predatory species should not have access to sugar gliders (38). Although people may perceive sugar gliders as safe within an enclosure, probably, smelling the presence of predatory animals, such as cats, in the same space will impact their mental state and restrict behavior.

6.1.2.3. Interactions with humans

Sugar gliders are nocturnal, so they should be handled at night when most active and not disturbed during daylight hours (38). Hand-reared sugar gliders handled quietly and calmly can develop into gentle companions (38). Scent has a vital role in social recognition in sugar gliders. For this reason, newly introduced and rehomed animals should be given time to recognize their handlers' scents (38).

6.2. Racing greyhounds housed in kennels

Greyhound racing is a sport and gambling industry sector that relies on small groups of greyhounds running competitively out of starting boxes on a racetrack at speeds of around 70 kilometers per hour. The distinct life stages of greyhounds bred to race typically involve breeding, rearing, early education, training, racing, and leaving the industry. However, the industry's practices have been subject to controversies and criticisms in the media and politics, with concerns about dog welfare and the business model's ethics (88, 89). Globally, commercial greyhound racing is declining, remaining legal only in the United Kingdom, Ireland, Vietnam, Mexico, New Zealand and parts of the United States and Australia.

TABLE 2 Examples of behavioral interactions (Domain 4) that can be provided to, and their utilization assessed in, sugar gliders housed in captivity (with aligned enrichment strategies from Table 1) that enable them to experience positive affective engagement (Domain 5) and their aligned agentic qualities.

Behavioral interactions	Agentic quality			
	Competence[1]	Choice[2]	Control[3]	Challenge[4]
Interactions with the environment				
A choice of materials[R] that stimulate foraging behaviors[A] (occupational, physical, nutritional)				
Aviaries of sufficient size[R] to allow gliding[A] (occupational, physical)				
A range of aviary items[R] to encourage scurrying[A], jumping[A], climbing[A], and gliding[A] (occupational, physical)				
Able to avoid items[R] in or near aviaries that may be perceived as a threat (sensory)				
Interactions with other animals				
Housed in groups of at least two individuals[M] to enable social interactions[A], and resting[A] (occupational, social)				
Nests of sufficient size[R] to allow individuals to huddle together[A] (social)				
Space[R] and housing design[M] that allows them to avoid[A] social interactions or predators that may be perceived as a threat (social)				
Interactions with humans				
Interactions limited to night-time only[M] (occupational and social)				
Frequent quiet and calm handling with control over their engagement with the handling[M] (social)				
Slow and controlled introductions to handlers[M] to allow scent identification and familiarisation[A] (social)				

Agentic qualities: [1]Characteristics and skills developed through opportunities to exercise agency; [2]Choice between two or more options; [3]Able to decide when and how to interact; [4]A variety of complex interactions that do not exceed an animal's current competency level. Types of animal welfare indicators: [R]Resource-based welfare indicators; [M]Management-based welfare indicators; [A]Animal-based welfare indicators.

Practices across life stages tend to follow the same general model. Pups are born and stay with their mothers until weaned. By 12 weeks, they enter the rearing phase, which may occur in a paddock, kennel or barn environment. During this stage, they are often housed with some littermates. They enter early education schooling at approximately 1 year as the starting point for training and chasing. They enter residential kennels where they are housed individually and participate in training, trials, and sometimes sales or amateur racing before starting professional racing around 15 months of age. Dogs continue to live in residential kennels until they exit the racing industry, usually by 5 years old, if not before. They may leave racing due to injury or death on the racetrack, being retired, rehomed as a companion, or transitioning to a breeding role.

One of the main controversies surrounding greyhound racing, aside from the high rate of injuries and deaths on the track (88, 90), is the inadequate housing conditions and lack of compensatory environmental enrichment. Another issue raised is the inadequate socialization of puppies which impacts their ability to adapt as companions in new homes later in life, along with the apparent overbreeding and euthanasia or unknown fate of dogs considered surplus, known as *wastage* (91).

Overall, the controversies and criticisms surrounding greyhound racing have contributed to growing public awareness and scrutiny of the industry internationally. This has increased pressure on regulators, stakeholders, and industry insiders to address the welfare and ethical issues raised and consider alternative models for managing and caring for greyhounds in the sport.

6.2.1. Domains 1 to 3

Greyhounds that race have increased nutritional demands (Domain 1). Nutrition should balance protein, fat, carbohydrate (including fiber), and vitamins. Protein is essential to support muscle use and growth. Extreme physical exertion likewise predisposes these dogs to dehydration (Domain 1). Inappropriate nutrition and hydration can lead to negative affective consequences such as thirst, hunger, weakness, and malaise of malnutrition. Appropriate hydration (Domain 1) is also necessary to control body temperature *via* panting (Domain 2). Systemic hyperthermia can result from exertion, hot environments, or an inability to cool effectively. Preventative health care is critical to optimize greyhound welfare (Domain 3). Disease prevention includes routine vaccination and parasite control. Training and racing intensity should match a dog's current physical competence level. This means consideration should be given to maintaining training during downtimes or rehabilitative training following recovery from injury/illness. The critical importance of racetrack-related environmental features (e.g., kennel facilities and catch pen design) and appropriate pre-race warm-up activities to reduce the incidence of injury are reportedly overlooked during race meets (92, 93).

6.2.2. Domain 4

Greyhounds that race spend a relatively brief period of their time budget running in one to two weekly races. Even if training, travel, handling, and kennelling are factored in, much of their time is spent outside engaging in racing-related activities. To counter the potential

for boredom or frustration in the intervening time and to build competence (94), greyhounds should be provided with opportunities to exercise agency. Examples of opportunities for positive welfare aligned with Domain 4 are further sub-categorized into interactions with the environment, other animals, and humans (Table 3).

6.2.2.1. Interactions with the environment

Designated spaces provided beyond the primary housing or kennel facility can allow greyhounds to explore and interact with their surroundings. Outdoor areas featuring a diversity of elements and substrates (e.g., grass, sand, trees, gravel, etc.) facilitate physical activities that promote fitness and allow for the expression of social (e.g., turning and jumping while engaged in social play) and other behaviors (e.g., digging) (95). Indoor spaces can be provided to preview the home environment (e.g., appliances and furniture) that retired dogs should transition to, allowing dogs to navigate and adapt to different challenges and settings that will set them up to succeed as competent animal companions beyond their time in racing (96).

Within their primary housing and transportation containment, sufficient space for easy stretching, lying down in full extension, and turning around should be ensured. This will enable greyhounds control to move comfortably. Providing multiple resting areas (e.g., elevated resting platforms and beds at ground level) allows dogs to choose how they utilize the space available to them (97). These provisions enable them to adjust their body positions, express their preferences, and exercise agency. Greyhounds may reposition bedding material to their liking, another way to exercise control. Providing more space to greyhounds promotes movement, reducing the likelihood they will experience affects such as frustration or discomfort. However, increased space alone is unlikely to offer sufficient agentic opportunities for positive welfare (98).

Interactive sensory stations can be provided in both indoor and outdoor spaces. These feature various scents, textures and objects for greyhounds to investigate and safely interact with. Based on their individual preferences and curiosity, such stations offer the dogs a choice as to what they engage with. Additional opportunities for positive experiences can come from devices such as puzzle toys and treat-dispensing toys, which engage greyhounds in challenge, both physically and cognitively (99). The complexity of spaces, objects, sensory stations and other novel objects should be gradually increased to support the animals' agentic choice and control to support the development of competence.

6.2.2.2. Interactions with other animals

Facilitating supervised interactions with other dogs allows greyhounds to develop and engage in appropriate social behaviors and establish positive social connections. Social connections provide opportunities for positive experiences through companionship, social bonding, and play (100–102). These experiences can also provide the greyhounds with exercise and a sense of comfort and security, promoting relaxation. Social housing, where compatible dogs live in pairs or small groups, facilitates social interactions. One way this can be achieved in a kennel facility is by enabling access between adjoining kennel runs so that multiple dogs can choose to be together or separate. Adequate space to comfortably accommodate the pair or group of dogs must be available in any kennel run if this strategy for shared housing is adopted.

Historically, greyhounds that race have been identified as having relatively poor socialization practices (103, 104). This can be related to isolated rearing occurring in rural locations and limited resourcing for active practices to adequately compensate. Social interactions with various other dogs help puppies learn and develop appropriate social and communication skills with conspecifics (105). Play groups that

TABLE 3 Examples of behavioral interactions (Domain 4) that can be provided to, and their utilisaton assessed in, racing greyhounds housed in kennels (with aligned enrichment strategies from Table 1) that enable them to experience positive affective engagement (Domain 5) and their aligned agentic qualities.

Behavioral interactions	Agentic quality			
	Competence[1]	Choice[2]	Control[3]	Challenge[4]
Interactions with the environment				
Sufficient space[R] to encourage free movement and play[A]				
Varied sensory inputs[R, e.g.,] nosework (olfactory-based sniffing activities[AM])				
Socialization and habituation[M] to common household environmental stimuli[R] to prepare for future rehoming as companion animals				
Interactions with other animals				
Access[M] to congenial relationships with other dogs, e.g., the choice[A] to live in pairs; regular play time[M] in small groups with compatible individuals				
Able to avoid threatening situations[A, e.g.,] sufficient space[R] and responsive monitoring[M] for threat avoidance				
Socialization[M] and habituation to other animals				
Interactions with humans				
Reward-based training[M]				
Positive interactions[M] with a variety of people[R]				

Agentic qualities: [1]Characteristics and skills developed through opportunities to exercise agency; [2]Choice between two or more options; [3]Able to decide when and how to interact; [4]A variety of complex interactions that do not exceed an animal's current competency level. Types of animal welfare indicators: [R]Resource-based welfare indicators; [M]Management-based welfare indicators; [A]Animal-based welfare indicators.

allow greyhounds to interact with other dogs of various breeds, sizes, ages and temperaments will expand their social skills' flexibility (i.e., competence) in response to dogs they meet throughout their life.

Positive experiences with other animals, both large and small, allow dogs to learn how to interact appropriately with different animals (106). This further develops their social skills and competence in multi-species environments, which is particularly relevant for successful rehoming following racing. Opportunities to interact with other animals can be provided with appropriate supervision and choice. In this way, individual dogs can exercise their agency, approaching and engaging with other animals (e.g., meeting a horse through a fence while on lead). Allowing greyhounds to learn to relate socially with other animals in a supportive manner is a challenge that can contribute to their overall competence. Foster programs in private homes (i.e., as often undertaken in working dog programs such as detection or guide dog rearing) during puppyhood and throughout the time a greyhound is racing may provide essential respite from the kennel environment (107) and alternative experiences to interact with a variety of animals and people (108, 109).

6.2.2.3. Interactions with humans

Ensuring that interactions with people, such as grooming and play sessions, are positive for greyhounds builds trust and promotes healthy attachment between the dogs and their caregivers (110, 111). For example, interactive play sessions between people and greyhounds can be undertaken using toys, agility equipment, or flirt poles. Such sessions enable the dogs to exercise choice in initiating and controlling their level of engagement while also challenging them physically and cognitively, promoting competence. Positive reinforcement training should form the basis of all foundational interactions between humans and greyhounds (112, 113).

Training activities can offer both cognitive and physical challenges relating to learning new behaviors, problem-solving, and overcoming obstacles of increasing complexity. With experience, this builds canine confidence in interacting with people, and their competence can increase. Dogs learn through every interaction that their behaviors directly influence the outcomes they receive, providing the individual animal with control in their training exercises. Greyhounds should be granted the choice to actively opt-out of training sessions if they do not wish to engage in the behaviors or with the equipment that will earn them rewards, providing them with control over their actions. Providing greyhounds with individual attention from people also allows for personalized interaction and the development of positive social bonds. This also facilitates the personalisation of training and care practices in a manner that can safeguard against fear, anxiety, or frustration.

It is important that greyhounds who race are able to meet a variety of people during puppyhood and their time in racing (114, 115). This include people of different ages, heights, appearances, and sex. Facilitating good socialization and ongoing experiences with a diversity of people allows greyhounds to interact positively (competently) with humans during and after their time in racing, a desirable trait for dogs.

6.3. Supporting agency and positive affective engagement

The two scenarios presented above are not intended to be exhaustive representations of how opportunities for agency could be supported in each. Instead, they have been used to illustrate how animals can be given opportunities to exercise agency in various contexts. Choice, control, and challenge represent agentic qualities that appropriate human care can provide, while competence likely results from these opportunities. Conversely, umwelt and affordances are agentic qualities not directly impacted by human care – so they have not been included in Tables 2, 3. They represent an animal's unique perceptual and effector world (umwelt) and their perception of what their environment offers them (affordances). Umwelt, affordances, and competence represent agentic qualities that need further exploration to identify potentially relevant positive welfare indicators.

While our evaluation of negative impacts in Domains 1 to 3 for each case study scenario focused on the potential mental experiences that might be inferred from conditions in each domain (e.g., hunger, weakness, and pain), this was not the case for Domain 4 (Behavioral Interactions) and positive welfare. Instead, we found it more beneficial to evaluate opportunities for agency to be exercised by considering agentic qualities of choice, control, and challenge that could be provided to the animal(s). In essence, we evaluated features of positive affective engagement (i.e., the collective term) rather than specific named positive mental experiences. This approach provides a means of systematically evaluating options to provide animals with opportunities to exercise agency. It may also help risk-averse animal welfare scientists cross the positivist-affective divide.

One flaw with our approach to evaluating positive welfare is that many behavioral interactions in our two scenarios mapped across similar or identical agentic qualities (Tables 2, 3). Therefore, detailed comparisons between interactions might be challenging to perform. An alternative approach might involve some indication of how strongly each agentic quality is exercised by a behavioral interaction being offered or occurring for the animal(s). For example, a behavioral interaction might offer an animal the ability to exercise a high level of choice, low control, and moderate challenge (Table 4). This behavioral interaction could then be compared against the agentic qualities of another interaction and this comparison might allow us to account for the interests of an individual animal or species. A non-numerical score could also be assigned to indicate how confident the rater is in assigning the strengths of these agentic qualities to the behavioral interaction (Table 4), i.e., to indicate the strength of the evidence used to assign the agentic score (116, 117).

Competence has not been included in Table 4 as this was the agentic quality that mapped across most behavioral interactions in our scenarios. The agentic qualities of choice, control, and challenge represent opportunities for agency that can be provided by human

TABLE 4 Opportunities for positive interactions (Domain 4) can be provided to an animal, and their utilization assessed so that the animal's experience of positive affective engagement (Domain 5) can be inferred.

Behavioral interactions	Agentic quality		
	Choice	Control	Challenge
Example behavioral interaction	**	***	*

The agentic qualities have been color-coded for each behavioral interaction being assessed. These colors represent how strongly each quality is exercised by the behavioral interaction being offered or occurring (e.g., green = high; yellow = moderate; red = low). Asterisk(s) could be used to indicate the degree of confidence a rater has in assigning the color code for each agentic quality – from low (*) to high (***).

care and management decisions, while competence is the potential result of these opportunities. Therefore, including competence did not provide additional information beyond that provided by the other three agentic qualities. However, future iterations could see competence included with sub-categories of physical and cognitive/mental competence to distinguish the types of competence that might result from each behavioral interaction (26, 34, 65).

In the two scenarios presented above, we have focused on opportunities for positive behavioral interactions. There is also scope to assess how well animals utilize these opportunities (15). An animal can be given opportunities to exercise agency (i.e., human care and management). Still, the animal's actual utilization of these opportunities determines whether or not they experience positive affective engagement (i.e., positive animal welfare). The approach in Table 4 might be used as a staged evaluation, where Stage 1 involves identifying opportunities for behavioral interactions, and Stage 2 is where the animal's utilization is assessed (15). However, animal utilization might be challenging to assess given that a lack of 'utilization' does not imply agency is not being exercised, i.e., an animal not interacting with an opportunity provided to them is still exercising agency through choice and control (57, 65). This area of evaluation and continuous improvement in offering greater agentic opportunities to animals under human care and management is an important consideration for future focus.

Future consideration should also be given to best practice communication with stakeholders (e.g., animal caretakers, industry bodies, regulators, policymakers, and the general public) about agency and positive animal welfare (118, 119). Translating theoretical and research findings to meaningful change for animals under human care often depends upon effective communication and subsequent human behavior change.

7. Conclusion

Animal welfare is a complex and multi-disciplinary field that encompasses the subjective mental experiences of animals. Focusing on mental experiences is becoming increasingly important in contemporary animal welfare science, as it aligns with other aspects of safeguarding and animal welfare assurance, such as ethics, policy, and laws. However, assessing animal welfare based on mental experiences can pose challenges, as they are subjective and cannot be directly measured. The concept of agency represents a new frontier in animal welfare assurance, as it allows us to consider how animals can be given opportunities to experience positive welfare by engaging in voluntary, self-generated, and goal-directed behavior that they are motivated to perform. This article argues that agency is a concept that straddles the positivist-affective divide and represents a way forward for discussions about and opportunities for positive animal welfare. Understanding the relationship between an animal's welfare and their ability to exercise agency can be illustrated through Domain 4 (Behavioral Interactions) of the Five Domains Model. Overall, the concept of agency provides a promising approach to understanding and improving the welfare of animals.

Author contributions

KL: Conceptualization, Writing – original draft, Writing – review & editing. MH: Conceptualization, Writing – review & editing. MC: Conceptualization, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

The authors would like to thank David Mellor for his helpful comments on an earlier version of this manuscript.

Conflict of interest

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Veterinary Science

TYPE Review
PUBLISHED 09 November 2023
DOI 10.3389/fvets.2023.1277512

OPEN ACCESS

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RECEIVED 14 August 2023
ACCEPTED 24 October 2023
PUBLISHED 09 November 2023

CITATION
Masebo NT, Zappaterra M, Felici M, Benedetti B and Padalino B (2023) Dromedary camel's welfare: literature from 1980 to 2023 with a text mining and topic analysis approach. *Front. Vet. Sci.* 10:1277512. doi: 10.3389/fvets.2023.1277512

Dromedary camel's welfare: literature from 1980 to 2023 with a text mining and topic analysis approach

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Dromedary camels are the preferable livestock species in the arid and semi-arid regions of the world. Most of the world's camel populations are managed under a subsistence/extensive system maintained by migratory pastoralists but intensification is getting more frequent. Even though recently the welfare of camels has been receiving more attention, in many countries there are no regulations to protect their health and welfare. The objectives of this article were to explore the main research topics related to camel welfare, their distribution over time and to highlight research gaps. A literature search was performed to identify records published in English from January 1980 to March 2023 on Dromedary camel welfare via Scopus®, using "Camel welfare," "Camel behaviour," "She-camel" and "Camel management" as search words. A total of 234 records were retained for analysis after automatic and manual screening procedures. Descriptive statistics, text mining (TM) and topic analysis (TA) were performed. The result shows that even though there were fluctuations between years, records on camel welfare have increased exponentially over time. Asia was the region where most of the corresponding authors were located. The first five most frequent words were, "milk," "calv," "behaviour," "femal," and "breed," the least frequent word was "stabl." TA resulted in the five most relevant topics dealing with "Calf management and milk production," "Camel health and management system," "Female and male reproduction," "Camel behaviour and feeding," and "Camel welfare." The topics that contained the oldest records were "female and male reproduction" and "camel health and management system" (in 1980 and 1983, respectively), while the topic named "camel behaviour and feeding" had the first article published in 2000. Overall, even though topics related to camel behaviour and welfare are receiving more attention from academia, research is still needed to fully understand how to safeguard welfare in Dromedary camels.

KEYWORDS

camelids, husbandry, production, machine learning, research

1. Introduction

The domestication of Camels started around 3,000 B.C. in South-East Arabia and South-West Central Asia (1, 2). The genus *Camelus* contains three species, the one-humped camels or Dromedary (*Camelus dromedarius*), the two-humped camels or Bactrian (*Camelus bactrianus*) (1) and the recently identified, never domesticated two-humped *Camelus ferus* located in the

Mongolian Great Gobi, in the Chinese Lop Nur, Taklamakan deserts (3). Usually, the Bactrian inhabits the northern colder areas and Dromedary is found in southern hotter areas of the old world. Dromedary camels (*Camelus dromedarius*) are found in different African and Asian countries (1) where they have primary economic, social, and cultural values (4). Dromedary camels are the main livestock species reared in the arid and semi-arid regions of the world where other livestock could not survive; their biological and physiological particularities enable them to withstand days in harsh environments with water and feed shortage (2, 5).

The world camel population is increasing continuously. In 2021, Chad, Somalia, and Sudan were the three countries with the largest camel populations, with 9.4, 7.4, and 4.9 million camels, respectively; it is estimated that the world camel population could reach 60 million in the next 25 years (6, 7). In the majority of nations, camel production is still a subsistence/extensive system, mostly maintained by migratory pastoralists in arid and semi-arid regions (8). Dromedary camels are multi-purpose livestock, used for carrying goods, in agriculture (ploughing and cultivation), as drought animals, for transportation and as a source of food (milk and meat) (1, 3, 8). In addition, in Middle Eastern countries, Dromedary camels are kept for sporting activities, such as camel racing, and for beauty contests (3). In recent years, there has been an increase in intensive camel production in peri-urban farms, supplying milk to urban dwellers (9). The growing intensification of camel husbandry systems is determined by the increase in demand for camel milk due to its nutritional and health enhancement benefits (10). The trend towards intensification in camel husbandry is also expected to increase in the coming years due to various reasons, including climate change (11). As a result of global warming, the temperature of the environment is increasing resulting in desertification, drought, and food shortages. Due to their adaptability and sustainability in extremely arid environments, Dromedary camels are therefore viewed with increasing interest even by countries where this livestock species was not traditionally bred (9). As interest in this animal species grew, so did the number of scientific works aimed at investigating its physiology (12), genetics (13), and welfare (14–16).

Animal welfare science has advanced rapidly in the last 30 years as a result of increased understanding of animal motivation, cognition, and the complexities of social behaviour (17). The methods employed in animal breeding, transportation and killing are subjects of public interest that lead to debates and activism (18). Meeting the rising demand for animal products without ignoring societal issues requires improving the efficiency of current animal production systems (19). Good welfare requires disease prevention, appropriate veterinary care, shelter, management and nutrition, a stimulating and safe environment, humane handling, and humane slaughter or killing of animals (20). There are various reasons for the growing demand for animal welfare enhancement, which is recognised globally through enaction of policies and regulations (21). Even though the attention given to welfare issues of Dromedary camels has increased in recent years (3) there are still no regulations establishing minimum requirements to protect the health and welfare of Dromedary camels (22, 23).

The concept of animal welfare has also changed a lot in the scientific field. Starting with the discussion of ethical positions, the concept of animal welfare has evolved (17), seeking a balance between public perception, and concepts of production, health, and psycho-physical well-being of animals. The term "animal welfare" can therefore be approached from different points of view and applied to different areas of study that are increasingly multidisciplinary. The identification of the topics most associated with animal welfare terms and their temporal changes provides a picture of this evolutionary process and suggests present and future trends. Bibliometrics analyses applied to literature allow for the screening of a vast number of records at both macroscopic and microscopic levels (24, 25). Text mining (TM) and topic analysis (TA) are extensions of classical bibliometric analyses and are machine learning-based techniques. These techniques are useful to investigate the trends in the scientific literature (26–28). By utilizing TM, it is possible to classify and group textual information, enabling the generation of outcomes like word frequency distribution, pattern identification, and predictive analytics that are not easily attainable with standard data analysis methods (29).

Therefore, this systematic review aims to evaluate literature dealing with Dromedary camel welfare that was written from January 1980 to March 2023 using TM and TA methods. This review was intended to improve understanding of topics associated with welfare of Dromedary camels, following their evolution through time and countries of publication, and to detect any gaps in knowledge and need for future research.

2. Methodology

2.1. Literature search

A systematic scientific literature search about Dromedary camel's welfare was performed to identify English records using Scopus® (i.e., the abstract and citation database for Elsevier®). The search was conducted on the 21st of March 2023. The keywords that were used for the search were included: "Camel welfare," "Camel behaviour," "She-camel" and "Camel management." Veterinary, biochemistry, genetics and molecular biology, social sciences, immunology, microbiology, multidisciplinary, neuroscience and engineering were included as the subject areas in the search. A Microsoft spreadsheet (Microsoft Excel®, v16.0, Redmond, WA, United States), which included all the records published from 1980 to the day of the search, was downloaded from Scopus®. In the spreadsheet, each line reported a record and each column the information extracted from the record such as: year of publication, authors, abstract, affiliation, country, regions, record type (e.g., article or review) and the source of publication (e.g., Journal title). The records were then screened and those that had no abstract, no author name, retracted or erratum, no source, or duplicates were excluded automatically. Finally, manual screening was performed by the researcher (MF) based on the topic and the species discussed in each record to decide the eligibility of the record for inclusion in the final analysis. In particular, records related to other species (e.g., Lama, Alpaca, ostriches) and other topics (e.g., socio-economic, infectious disease) were excluded. Records that studied Dromedary and/or Bactrian camels in combination with other livestock (e.g., buffaloes, cows, goats etc.) were retained. Records that were difficult to categorise were checked by a welfare expert (BP), who made the final decision on whether they should be excluded or included in the study. The screening process is further summarised in a flow chart indicating all the steps with the number of records excluded or retained in each step (Figure 1). Descriptive statistics

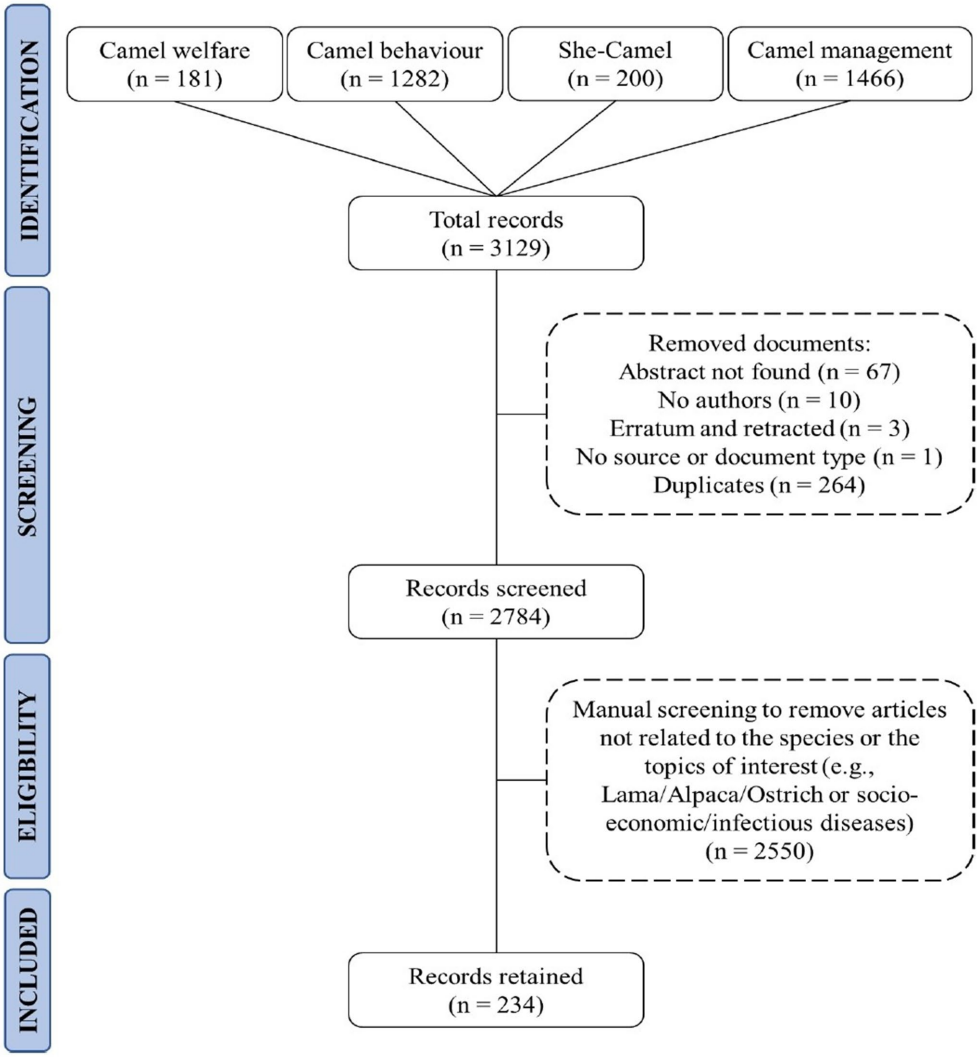

FIGURE 1
Flow chart of the scientific literature showing the number of records discovered with each keyword sequence and the total number of records included in the review of camel welfare literature.

based on the regions of origin of the records, countries, and year of publication were performed using Excel Pivot tables and results are presented as graphs. The regions of origin of the records were identified based on the affiliation of the corresponding author and, if not indicated, of the first author.

2.2. Text mining

An additional Excel sheet was prepared containing two columns namely "ID" and "abstract" of the records for TM analysis. The authors standardised the corpus of records using only British English, as some words in the corpus were spelt both in American and British English. In particular, the handling process was performed on the word pairs "behaviour"-"behavior," "analyse"-"analyze," "program"-"programme." Therefore, TM analysis was performed on the abstract of the records that were retained for the final analysis (26). The corpus of records was submitted to pre-processing steps according to Sebastiani (30). In detail, the text was reduced to lowercase, and unusual symbols (e.g., "@," "/" or "*"), punctuations, numbers, and English stop words (e.g., "the," "a," "and," "on," "at") were removed. In addition, researchers removed words strictly associated with the search or commonly used in scientific articles, namely "camel," "camels," "group," "groups," "test," "time," "significantly," "significant," "significative," "significance," "study," "studies," "she," "animal," "animals," and "management." At the end of these processes, the extra spaces within words were removed. Text tokenization was performed to reduce words to their root. The next step was to create a document-term matrix (i.e., a matrix that contains the records along the rows and the terms along the columns) as reported in the literature (26). In order to identify the weight of each word, a term frequency-inverse document frequency technique (TFIDF) was applied (31). This is the frequency of a term adjusted for how extensively it is used, demonstrating the importance of a word in the overall collection of records (27). In this study, as reported in the literature (27), the first set of 25 words was presented as a histogram. In our corpus of records, to obtain these 25 words, the TFIDF cut-off

was set to 1.96, which represented the weight of the 25th word. A cloud of the most relevant words (TFIDF ≥1.96) was also constructed using the website,[1] with larger character sizes indicating a higher TFIDF value. Associations among the most relevant words (TFIDF ≥1.96) and all the record terms were identified, using a grade of correlation ≥0.3. To calculate associations, the frequency with which two words emerge together was considered. Particularly, if two words always emerge together the association is 1 and if they never emerge together the association is −1. The TM analysis was carried out in R environment (32) using functions from the package's "tm," "SnowballC," "ggplot2," "dplyr," and "tidyverse."

2.3. Topic analysis

In order to perform TA, Latent Dirichlet Allocation (LDA) approach was applied (33). LDA is a hierarchical Bayesian technique that learns a set of theme topics from words that appear together frequently in records. A single subject can be thought of as a multinomial distribution of words, and a single record as a multinomial distribution of latent topics. The model infers the hidden topic structure from the observed records and words, generating per-record topic distributions and per-topic word distributions (33). LDA function with Gibbs sampling option of the "*topic models*" package in R was used (34), and the R library "*tidytext*" was used to present the graphic of the commonest words of each topic and their relative probability to belong to that topic (beta value). Before TA commenced, the number of topics in which the corpus had to be split was determined. However, because the "ideal" number is generally unknown, trials with 5, 6 and 8 topics were performed and the most suggestive panel among them was chosen based on consensus among the researchers. Once the definitive number of topics ($n = 5$) was identified, each researcher independently named them providing an indicative label. The final label of each topic was discussed and defined with the agreement of all researchers. To classify the topics, the cumulative probabilities (cp) of the first 20 words of each topic were calculated. Topics were shown according to this classification (i.e., topic 1 has the highest cp).

3. Results

3.1. Descriptive statistics

Out of 3,129 abstracts that were downloaded from Scopus®, 234 (7.45%) fulfilled the screening and eligibility criteria and were retained. Not pertinent [i.e., about other species, other topics such as socio-economic topics etc. (81.49%)] was one of the main reasons to exclude records from further analysis. The other most frequent reasons for exclusions were the following: duplicates (8.44%), no abstract (2.14%) and no author found (0.32%) (Figure 1). The type of records retained were research articles (205/234; 87.6%), reviews (14/234; 5.98%), book chapters (9/234; 3.85%), conference papers (3/234; 1.28%), notes (2/234; 0.85%) and books (1/234; 0.43%).

The total number of records published per year has increased exponentially over time (Figure 2). Based on the corresponding author address, India (31/234; 13.25%), Pakistan (19/234; 8.12%), United States of America (17/234; 7.26%), Italy (17/234; 7.26%) and Egypt (13/234; 5.56%) were the countries from which most articles were submitted (Figure 3). Asia (37.17%) was the region where most of the corresponding authors were based followed by Europe (25.64%) and Africa (23.5%) (Figure 4). The records were published in 87 different scientific journals (Supplementary material S1).

3.2. Text mining

After pre-processing of the data and reduction of sparseness (i.e., exclusion of the "rare words"), 1,346 terms were retained from the selected 234 records. The most relevant words (TFIDF ≥1.96), according to the TFIDF ponderation system, are represented in a histogram (Figure 5) and a word cloud (Figure 6), with the font size proportional to the TFIDF of each word. The words with the highest TFIDF were "milk" (5.71), followed by "calv" (3.97), "behaviour" (3.37), "femal" (2.70), "breed" (2.65), "product" (2.63), "system" (2.62), "welfar" (2.59), "male" (2.47) and "feed" (2.46). The word with the lowest TFIDF was "stabl" (0.1). The associations between the most relevant words (TFIDF ≥1.96) and the other words of the matrix are shown in Table 1. The words "female," "Breed," "Feed," "Season," "Concentr" and "Level" showed no significant correlation (with correlation grade ≥ 0.3) with other words.

3.3. Topic analysis

Five topics were chosen as the ideal topics and labels were assigned to each of them. The name of each topic as well as the number of records contained in each topic are shown in Table 2. Figure 7 shows the topics numbered from 1 to 5 according to the cumulative probabilities (cp), and the first 10 words for each topic. The topics containing the oldest records were those named "female and male reproduction" and "camel health and management system" (in 1980 and 1983, respectively), while the topic named "camel behaviour and feeding" were contained the most recent records, published in 2000 (Figure 8). The TA performed with 6 and 8 *a-priori* numbers of topics are shown in the Supplementary material S2.

4. Discussion

Performing a literature review is a crucial approach to analyse the present status of a specific topic and offer guidance for future research directions (33). This systematic review performed using the statistical methods of TM and TA yielded valuable information about the welfare of Dromedary camels from a vast collection of scientific literature written over the last four decades. These techniques enable the authors to evaluate diverse themes in the subject area and identify gaps in knowledge.

The number of records on Dromedary camel welfare has increased exponentially over the years. This was expected, because animal welfare research, as an interdisciplinary field of research that started to develop in the 1970s, has gained prominence since then. The

1 www.wordclouds.com

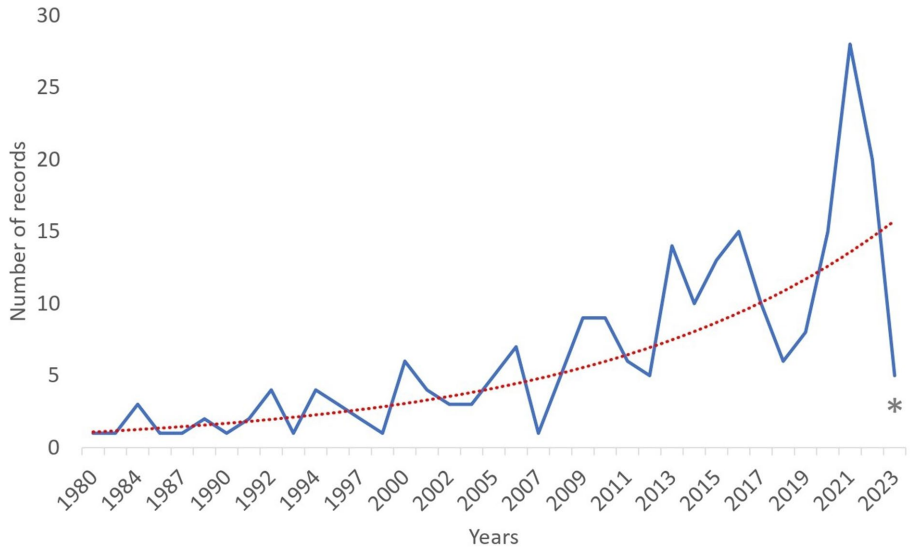

FIGURE 2
Number of records distributed by publication year (1980–2023) of 234 records selected for inclusion in the review. The exponential trend is represented by the dashed red line. * Indicates that results in this year are related to the period from January to March.

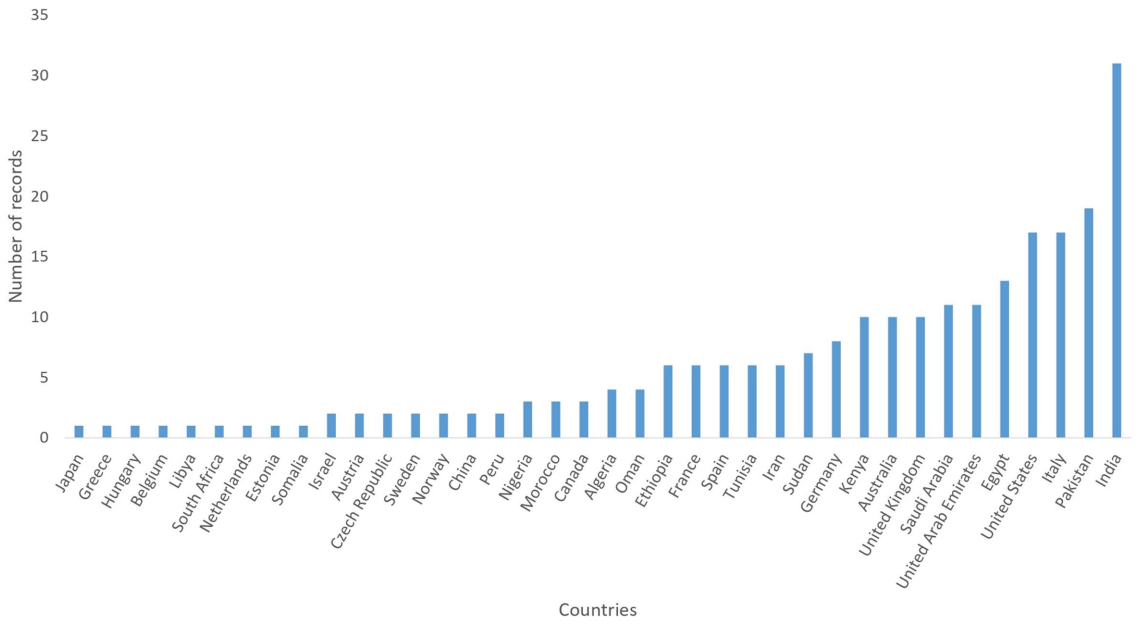

FIGURE 3
Number of records based on countries of the 234 records selected for inclusion in the review. The countries are based on the nationality of the corresponding authors.

driving force has been public concern about the welfare of animals kept in different husbandry systems (35). Additionally, animal welfare and social and environmental sustainability are also becoming more and more significant (19). Therefore, the increasing concern for animal welfare and a growing belief that farm animal welfare should be protected and improved (36), coupled with the recognition of the unique challenges faced by Dromedary camels, has driven a rapid rise in the number of records addressing their welfare (3). Findings reported here show that there is a high number of records, and the number has steadily been increasing particularly from 2020. According to recent bibliometric research by Kandeel et al. (37), the year 2020 marked a highly productive year for camel research. The authors suggested that the recent surge in camel studies could be attributed to the availability of an increased number of records and special issues specifically focusing on camels and their role as natural reservoir species for respiratory virus outbreaks, such as MERS-CoV infection. Furthermore, this remarkable increase in records has also been driven by recent international projects and collaborations, such

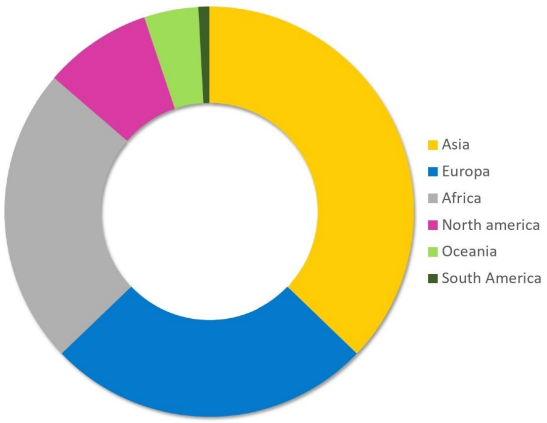

FIGURE 4
Distribution of records based on regions. The regions were determined based on the nationality of the corresponding authors.

as the *CA*.RA.VA.N network (towards a CAmel tRAnsnational VAlue chaiN; https://anr.fr/Project-ANR-16-ARM2-0002) and the International Camel Consortium for Genomic Improvement and Conservation[2] running in those years.

Most of the records on camels came from Asia; this is not surprising considering that Asia has the second-largest Dromedary camel population globally (6). Furthermore, the intensification of Dromedary camel production has been on the increase in Asian countries (9–11), and these nations boast dedicated research centers focused on camel research (38). Similarly, Iglesias Pastrana et al. (39) in their bibliometric research about camels indicated that countries with traditionally well-established camel farming are responsible for the papers with the highest academic impact. However, in our findings, Europe emerged as the second-leading region in terms of published research, despite having a relatively small population of Dromedary camels. This achievement can be credited to collaborative research conducted between European researchers and experts from traditional camel-rearing countries (38). Several European countries, such as France, Germany, Spain and Italy, have often been involved as partners in research projects with Africa and Asia. International research projects and collaborations on camels have largely benefited from the inclusion of research teams from African and Middle Eastern countries with well-established traditional camel breeding and production systems as partners (39). However, researchers working in more advanced countries or research centers with a long history of recognised scientific expertise often play important roles in coordinating or directing these international projects, which may explain why, based on the address of the corresponding authors, Europe is the second geographical region publishing on camel welfare (39). It is also possible that the significant funding support provided for camel research by the European Commission (EC) (37) could have contribute to increased scientific interest towards this livestock species. Finally, the growing interest in camels shown by various Western countries, such as Italy, can also be explained by the interest raised by this species from a climate change perspective. Dromedary camels are seen as one of the most sustainable livestock species due to their ability to produce even in arid and extreme environments (3, 11); this feature is seen with increasing interest from Mediterranean countries, where summers are increasingly arid (40).

Despite the impact of climate change on water and food resources, the world demand for animal sources products is rising, particularly in developing nations (41). As a result, the demand for camel and goat milk is estimated to triple by 2050 in different African regions (42). Achieving adequate animal welfare might be crucial for increasing the production and safety of animal products to satisfy the demands of the consumer (19). It is therefore not surprising that the first five most frequent words with the highest TFIDF were "milk," "calve," "behaviour," "female," and "breed." Camel milk acceptance and commercialization have increased over the years, and it is being used as treatment for chronic disease conditions like diabetes and peptic ulcers (43). Dromedary camel milk is similar to human milk, and its lower-calorie content makes it ideal for persons with diabetes or obesity (23). Nowadays the Dromedary camel milk market has increased, making camel production more specialised in dairies, and leading to the advancement of camel milk production (3). The occurrence of "calve" as the second most frequent word was as expected, given that most of the scientific literature addresses dairy camels, and as camel dairy farms become more intensive, calves are moved away from their mothers (11). Much attention is therefore needed in the management practices of calves, prioritizing the identification of management strategies for the improvement of calf health and welfare. From the articles retained and analysed it is evident that a lot of attention has been placed on the growth performance and welfare of calves in different camel management systems, such as semi-intensive and traditional camel husbandry systems (44, 45). "Behaviour" was also a term frequently associated with camel welfare. In general, animal behaviour is a highly frequent topic of investigation in animal welfare. Researchers examine behaviour under various conditions to determine behavioural patterns and responses. Similarly, in camels, the retrieved studies explored how camels behave in different housing setups and environments, during husbandry and reproduction, and while feeding. The purpose is to evaluate the welfare of camels and gain insights into how their behaviour changes under different circumstances and how behaviour can be used to assess their welfare condition (16, 46–48). As with other animals, camelids do have behavioural needs that must be met to ensure their welfare. These include the possibility to express species-specific behaviours, prevent illnesses, and live in a suitable social setting (23). Overall, the TM analysis picked the most frequent words associated with Dromedary husbandry, management, milk production, calf management and welfare.

This review highlighted the prominence of welfare-related studies in dairy camels. According to the cp statistical analysis of the topics, the most important was "Calf management and milk production" (topic 1). The articles selected for inclusion in the analysis reflect a strong scientific emphasis on calf management and the enhancement of calf welfare in different camel husbandry systems through the evaluation of behavioural and physiological indicators, with the objectives of producing camel milk without affecting calve performance, health, and welfare. Additionally, camels are social,

2 www.icc-gic.weebly.com

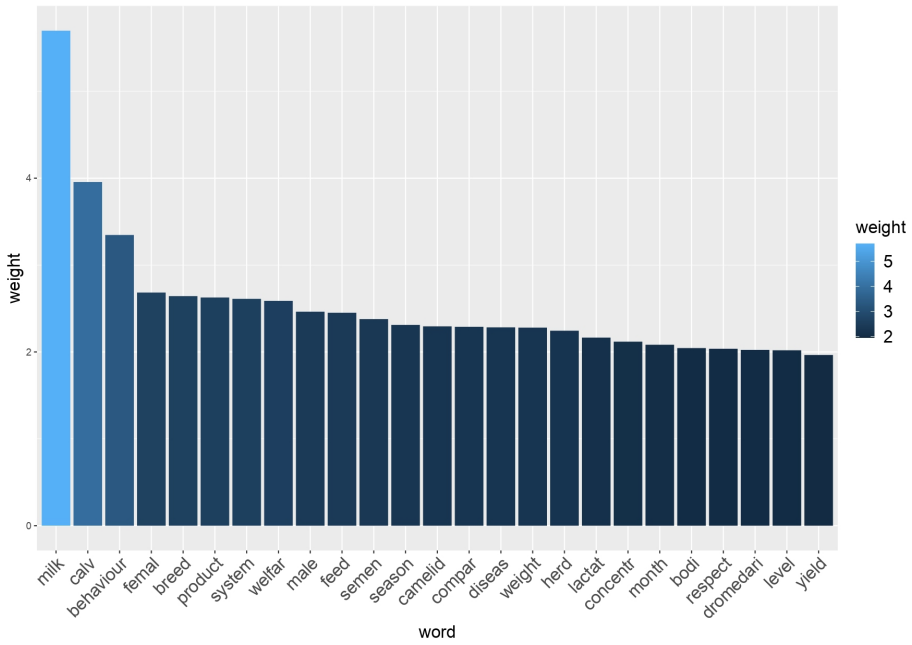

FIGURE 5
Histogram showing the most relevant words (TFIDF ≥1.96) of 234 records selected for inclusion in the study and their respective weights.

FIGURE 6
Word cloud representing the most relevant words of the corpus of 234 records selected for inclusion in the review. The size of the words is proportional to the weight they have in the corpus.

calm, and peaceful herd animals with close bonds among themselves and with their offspring. Physical and visual contact with the calf is essential for milk production to continue (49), and this could explain the frequent occurrence of the words "milk" and "calv" in the LDA analysis. Until recently, despite camels being a significant food source in arid and semiarid regions, their milk production potential has not been exploited. Dromedary camels have long lactation periods and can produce milk even in times of feed shortage, making this animal important for attaining food security and a source of income (22). The lactation length in camels typically spans around 12 months, but it can vary, ranging from 9 to 18 months (50). Milk production is influenced by a variety of factors, predominantly encompassing genetics, age, parity, lactation stage, nutrition, management, calving month, and day length (51, 52). Nonetheless, the specific impact of these elements on camel milk production remains inadequately explored, and our comprehension of their physiological processes in this context is limited (53). Historically, camel milk was solely obtained through manual milking practices within traditional, extensive, or semi-intensive farming systems. The milk was primarily consumed locally, with limited processing, and only a small portion of the production made its way to urban markets (11). However, under favourable circumstances, intensive production is performed and can present several benefits. It facilitates the efficient and economical production of quality grade raw camel milk, well-suited for subsequent processing, meeting the discerning quality demands of modern consumers. Simultaneously, this approach ensures compliance with the camels' health and welfare needs, adhering to national and international guidelines, statutory requirements, and industry standards (11). Therefore, with the surge in global demand for camel's milk and the consequent shift towards modern, industrial camel milk production (3), research interest in camel milk and production has increased, this may be one of the factors making this the first area of research.

The second most important topic was "Camel behaviour and feeding" (topic 2). This observation demonstrates the broad scope of behaviour-related topics in camel research, encompassing areas such as feeding behaviour, seasonal behaviour in relation to reproduction, and welfare studies. In recent times, the husbandry practices for Dromedary camels have been transitioning towards a semi-intensive system. This shift is influenced by changes in the animal's role and the settlement of nomadic populations. However, this move towards captivity can potentially lead to limitations in the expression of

TABLE 1 Associations between the most relevant words (TFIDF ≥1.96) and the other words present in the corpus of 234 records selected for inclusion in this review.

Words (TFIDF ≥ 1.96)	Words associated (grade of correlation ≥ 0.3)
Milk	Udder (0.55); machin (0.53); letdown (0.45)
Calv	Interv (0.52); first (0.51); februari (0.42); open (0.41)
Behaviour	Sexual (0.68); heat (0.63); induc (0.57); habitat (0.55); specif (0.47); adapt (0.44); natur (0.40)
Product	Meat (0.42)
System	Semi-inten (0.64); Khartoum (0.40)
Welfar	Buffalo (0.4)
Male	Intromiss (0.44); mount (0.41)
Semen	Collect (0.73); artifici (0.62); sperm (0.61); ejacul (0.53); insemin (0.46); preserv (0.44); modif (0.43); resili (0.42)
Camelid	World (0.59); american (0.56); south (0.51); suscept (0.49); metabol (0.45); chapter (0.44)
Compar	Quit (0.69); allot (0.66); biometr (0.61); khejri (0.58); prosopi (0.58); less (0.57); iron (0.55); zinc (0.55); cost (0.53); trial (0.53); manger (0.49); copper (0.48); inten (0.48); wither (0.46); hind (0.45); random (0.45); total (0.43); gain (0.41); economy (0.40)
Disea	Origin (0.55); scope (0.48); introduc (0.47); togeth (0.46); difficulti (0.45); interpret (0.45); infecti (0.42); worm (0.41)
Weight	Gain (0.58); birth (0.52); growth (0.45)
Herd	Mortal (0.42)
Lactat	Fourth (0.63); highest (0.56); pariti (0.55); composit (0.48); peak (0.47); similar (0.46)
Month	Januari (0.42); februari (0.40)
Bodi	Circumf (0.50); quit (0.47); allot (0.44); trial (0.41)
Respect:	Eight (0.47); parturit (0.47); symptom (0.45); newborn (0.42)
Dromedari	Recent (0.43)
Yield	Highest (0.58); composit (0.56); pariti (0.56); peak (0.47); record (0.45); persist (0.44); similar (0.41)

The correlation grade is written between the brackets. The grade of correlation was set at ≥0.3.

TABLE 2 Numbers and labels of the 5 topics revealed with LDA analysis of 234 records selected for inclusion in the review and number of records included in each topic.

Number of the topics	Label of the topic	Number of records per topic
1	Calf management and milk production	55
2	Camel behaviour and feeding	39
3	Camel welfare	54
4	Camel health and management system	50
5	Female and male reproduction	36

The final labels of the topics have been chosen with the agreement of all researchers.

various behavioural needs, impacting the camels' social activities and leading to the manifestation of stereotypic behaviours (47). Animal behaviour is strongly influenced by the surrounding environment, and behavioural modifications serve as valuable tools for assessing the effects of different management approaches on animal welfare. Although they share many characteristics with ruminants, these animals are taxonomically, anatomically, physiologically, and behaviourally distinct, meaning that they have separate needs (23). Behaviour, health, pathology, productivity, and animal welfare are intricately interconnected. Therefore, behavioural problems serve as vital indicators of compromised welfare in these animals (14).

Until recently, the welfare of camels has not been prioritised (11). However, interest in this topic has increased enormously, so much so that the third statistically (cp) most important topic identified through LDA analysis was "Camel welfare" (topic 3). Although scientific interest in animal welfare has grown significantly as a result of consumer concern worldwide, it is still disregarded in some species, such as farmed camels. To maintain ethically acceptable conditions in these animals while they are reared, evidence-based parameters evaluating environmental and animal-based welfare indicators and scores must be established (21, 39). Animal welfare studies can provide information on the circumstances that might promote excellent welfare (54). The currently available protocols have been developed for intensive, more or less industrial, systems in developed countries. However, the principles of Welfare Quality® can be used to identify animal welfare issues and risks in all systems (55). A recently published protocol for the assessment of Dromedary camel in intensive and semi-intensive systems (14, 15) adapted Welfare Quality and AWIN protocols to this species. However, the latter protocol is not useful in extensive, pasture-based systems and small, traditional farms in developing countries because of the different characteristics of the production units (19), and needs, therefore, future adaptation and validations. Moreover, improving animal welfare means ensuring that the animal experience is as positive as possible, which often requires changes in the infrastructure and practices of those responsible for the care and handling of animals (56). So, much more work is needed to understand how to measure welfare, and in particular, positive welfare, in Dromedary camels.

A crucial aspect related to animal welfare is animal health, as highlighted by the fourth most important topic identified in this review (i.e., topic 4, named "Camel health and management system"). Animal health and animal welfare are complementary but not synonymous. Without good health, there cannot be good welfare, but good health alone does not guarantee good welfare (21). In the past, camels were thought to be resistant to diseases; however, this belief is no longer accurate (57). Currently, numerous viral, bacterial, and parasitic diseases affecting camels have been well-characterised (57–59). Diagnoses of these diseases are now frequently and accurately made in semi-intensive and intensive camel farming (60). However, most camel populations are managed under pastoralist nomadic environments, and in these nomadic pastoral communities, it is hard to adhere to animal health standards used in Western livestock systems (61). It is therefore important to enhance the veterinary health services also in those areas, to ensure the principle of good health.

"Female and male reproduction" (topic 5) was the fifth most important topic identified. It is critical to ensure sustained high levels of reproduction in camels, not only for profitable production but also

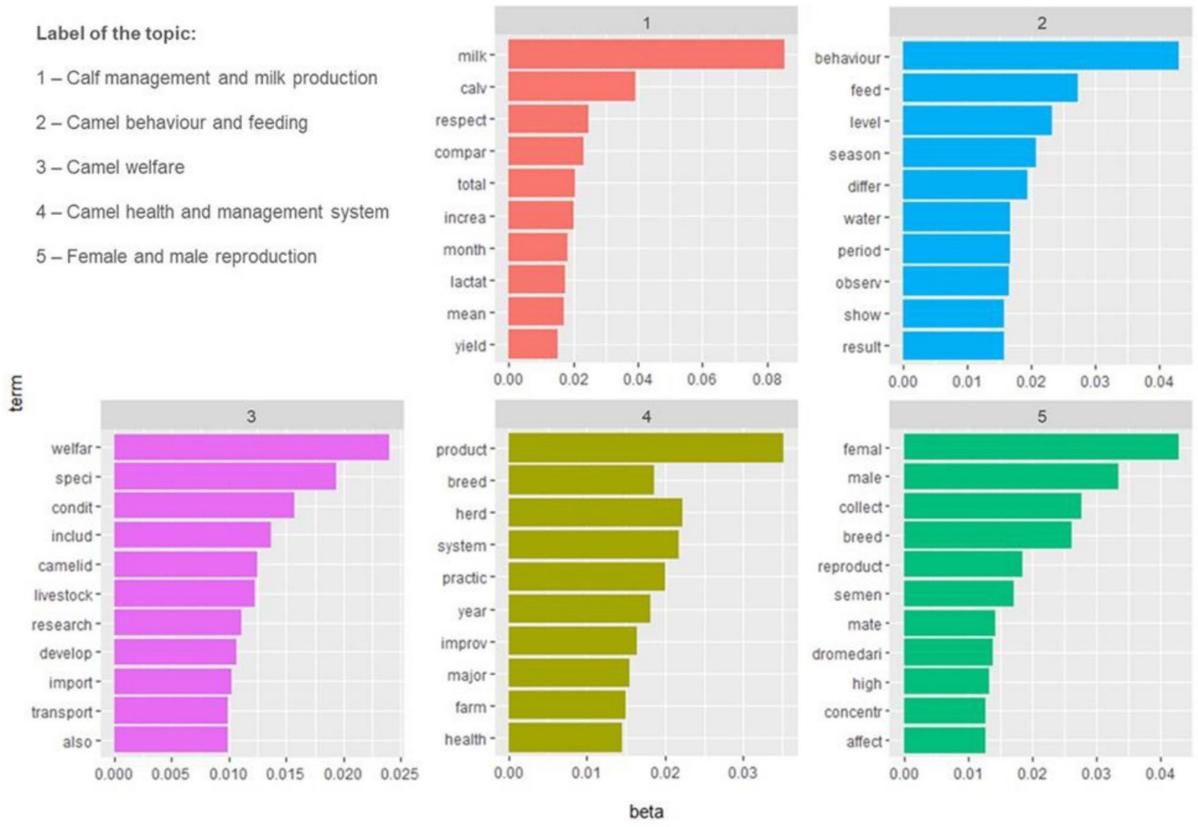

FIGURE 7
Histograms showing the ten most frequent words within the five topics revealed with LDA analysis of 234 records selected for inclusion in the review. Beta indicates the relative probability of each term belonging to that topic. The topics were ordered from 1 to 5 in accordance with their cumulative probabilities.

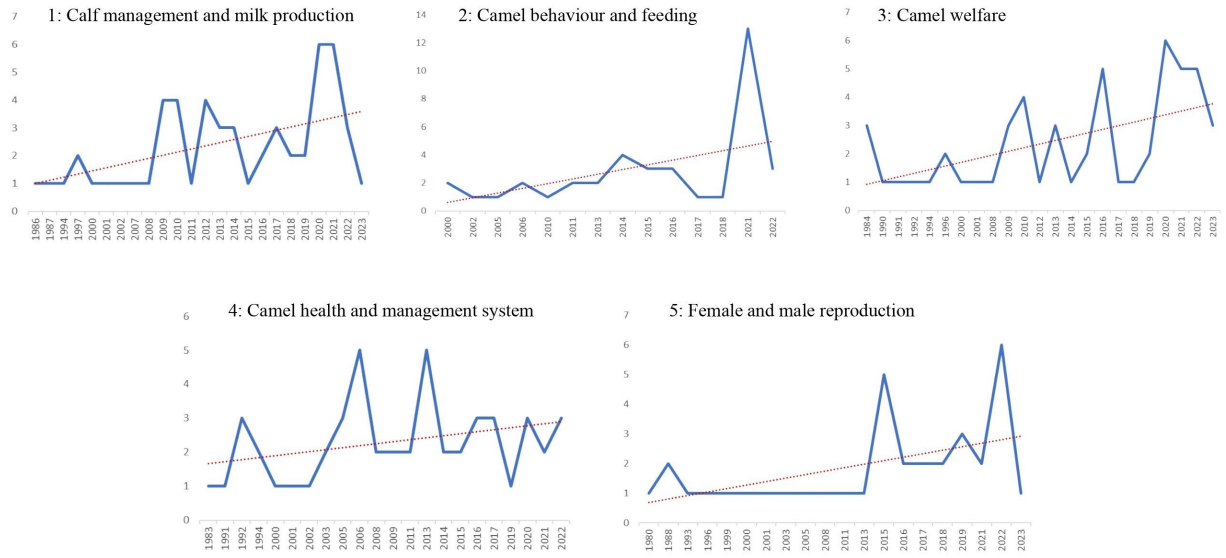

FIGURE 8
Number of records included in each topic starting from the year of the first publication. The results for 2023 are for January through March.

to provide abundant possibilities for selection and genetic improvement (62). Despite an increase in camel production, in different Asian and African nations they are still managed under the traditional system records, making it difficult to implement genetic

improvement (63). To exploit the full potential of camels, genetic improvement is essential and artificial insemination is highly needed (64). In fact, through artificial insemination, it is possible to prevent the spread of venereal diseases and allow the genes of highly valuable bulls to be spread more widely. However, the implementation of semen collection and artificial insemination is still problematic in Dromedary camels (65). Camels are seasonal breeders (66), and during breeding season males become restless and aggressive (22). To prevent aggressions, bulls are often individually stabled, and movement restriction, reduced space and lack of social contact can lead to stereotypical behaviours and impaired welfare (47, 67, 68). The collection of semen is done using either electro-ejaculation or an artificial vagina (AV) (65). The collection of semen utilizing electroejaculation is a welfare concern and not recommended since it requires the use of sedation or anaesthesia and is life-threatening, furthermore, the amount of sperm collected by this technique varies greatly (65). Currently, despite the large gap with other livestock species, efforts are being made to improve and make extensive usage of assisted reproductive technologies to improve the reproductive efficiency of camels, such as embryo transfer and artificial insemination (11). However, more work is needed to implement welfare-friendly reproduction techniques.

The limitations related to the method used to realise the present literature review must be reported. Firstly, synonyms of the words used in the search strings may have not been considered, leading to a reduction in the number of records that could have been included. Secondly, records not included in Scopus® were not considered, and the same was for the "grey literature," which is not included in Scopus. Furthermore, parameters of the search, such as the English-only language of the abstracts or specific subject areas, and the screening criteria adopted may have reduced the number of records analysed. Finally, the method of analysis used in the present review implied that the 234 records were not fully read but considered only from the title and abstract. Notwithstanding these limitations, this study reviewed the literature related to camel welfare, identifying the leading topics of camel scientific research and the gaps in knowledge about this animal species.

5. Conclusion

Through the utilization of text mining and topic analysis techniques, this review has identified and emphasised the most frequently investigated topics in Dromedary camel research related to animal welfare. Additionally, this study has shed light on the areas of camel welfare that remain unexplored and in need of further research. The result also indicates that there is exponential growth in the literature on Dromedary camel welfare. A higher number of records come from those countries where there is a growth of Dromedary camel populations and from traditional camel-rearing countries in Asia. The LDA identified the most important topics dealing with aspects of husbandry, management and welfare of Dromedary camels, milk production and calf management, behaviour and feeding management, camel welfare, camel health, and management system, and heading to female and male reproduction. Moreover, this review shows that although camel behaviour and welfare have received more attention recently from academia there is a need for more research to help improve our understanding of the welfare-related issues of Dromedary camels. Lastly, despite the limitations, this review gives an overview of the landscape of the camel welfare literature, highlighting both the most widely covered topics and those that still need in-depth study by scientists around the world.

Author contributions

NTM: Data curation, Investigation, Writing – original draft. MZ: Conceptualization, Investigation, Methodology, Writing – original draft. MF: Conceptualization, Data curation, Investigation, Methodology, Writing – review & editing. BB: Conceptualization, Investigation, Methodology, Resources, Writing – original draft. BP: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was funded by Animals' Angels.

Acknowledgments

The paper was born within the framework of the programmatic initiatives of the ASPA Commission for Animal Behaviour and Welfare, and the authors are thankful to Dr. Barbara Cortiero who taught these machine-learning techniques to the members of the Commission.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1277512/full#supplementary-material

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frontiers | Frontiers in Veterinary Science

TYPE Original Research
PUBLISHED 12 February 2024
DOI 10.3389/fvets.2023.1335484

OPEN ACCESS

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RECEIVED 09 November 2023
ACCEPTED 22 December 2023
PUBLISHED 12 February 2024

CITATION
Yan S, Li H, Lin J, Chen H, Liu S and Diao H (2024) An investigation of the perceptions of laboratory animal welfare issues among undergraduate and graduate veterinary students in southeastern China. *Front. Vet. Sci.* 10:1335484. doi: 10.3389/fvets.2023.1335484

An investigation of the perceptions of laboratory animal welfare issues among undergraduate and graduate veterinary students in southeastern China

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Animal experiments have played a significant role in advancing scientific knowledge and enhancing people's quality of life. In order to better understand the opinions and knowledge of veterinary students in the domain of laboratory animal welfare and to explore and advance the teaching methods used in animal ethics education, a questionnaire was designed and used to conduct a survey among undergraduate and postgraduate students majoring in veterinary medicine. The survey encompassed various topics, such as students' level of knowledge about animal welfare, their perspectives on laboratory animals, their proficiency with animal experiments, and their opinions on teaching methods and content. The respondents were a total of 150 undergraduate students and 148 graduate students. The survey results indicated that most students expressed a strong sense of responsibility for the safeguarding of the welfare of experimental animals. However, there were a few students who lacked compassion for animals. Additionally, there was a general lack of basic theoretical knowledge of animal ethics and an inadequate grasp of experimental techniques among current students. Furthermore, most of the participants expressed a strong sense of responsibility to advocate for animal welfare. Although a substantial number of students were unaware of the existence of agencies for the supervision of work involving laboratory animals, they supported teaching and supervision in the domain of animal welfare and were open to various teaching methods and topics of content. In conclusion, targeted training and education regarding laboratory animal welfare and ethics should be conducted in the future to address the specific needs of students. This study provides a foundation for future animal welfare education and will help to improve the professional skills and humanistic qualities of veterinary students.

KEYWORDS

Chinese veterinary students, animal welfare, laboratory animal, university education, survey research

1 Introduction

In recent decades, there has been remarkable worldwide advancement in the understanding of diseases and their diagnosis and treatment. This progress is largely attributable to the introduction and utilization of laboratory animals. Animal experimentation represents one of the primary methods used to discover new knowledge in biomedical science, with experimental animals consistently playing a vital role in the new stages of the rapid development of research in precision medicine in China. Through animal experiments, researchers can delve deeply and comprehensively to explore the mechanisms underlying the pathogenesis and progression of human or animal diseases (1, 2). Additionally, animals have been utilized to enhance our understanding of animal and human anatomy and physiology, as well as to assess the safety and efficacy of drugs and vaccines (3–5). In short, experimental animals are indispensable in multiple areas of life science and have made significant contributions to the advancement of scientific knowledge and enhancement of people's quality of life. Although most people understand and appreciate the need for animal-based research, the welfare of laboratory animals and the ethics of their use has become a recognized issue and widespread concern worldwide (6–8).

The progress of society and national development in quality of life have led to a paradigm shift in the perception of animals. Animals are no longer considered mere extensions or tools of human beings, but rather are regarded as independent beings with the inherent right to life (9, 10). This recognition of the importance of animal welfare and a growing awareness of the principles of animal rights have resulted in a heightened emphasis on the ethical considerations associated with the use of experimental animals (11). Consequently, increasing amounts of attention are being paid to the ethical issues surrounding experimental animals.

Ethical issues surrounding experimental animal research extend beyond the wellbeing of the animals themselves; they also impact the scientific integrity of experimental outcomes (12). Furthermore, these ethical considerations have implications for the quality of education and experience with scientific research for veterinary students. It has been demonstrated through various studies that the attitudes of researchers toward laboratory animals can be influenced by a range of psychosocial factors, including their level of education and training (13, 14). In order to obtain reliable and effective experimental results, it is crucial to prioritize the welfare of laboratory animals, which is directly dependent on the actions of the experimenter (15). As a result, university education plays a pivotal role in enhancing the theoretical knowledge and practical skills of veterinary students in the domain of laboratory animal welfare and ethics, enabling them to ensure that the welfare of laboratory animals is maintained and their needs guaranteed to be met in a timely and effective manner.

Despite the emphasis placed on animal welfare by leading veterinary organizations in China with well-defined standards, unfortunately there exists a clear disparity between the southeast regions and "first-tier" cities like Beijing in terms of veterinary student education and training in animal welfare (16).

The purpose of this study was to assess current perceptions and understanding of laboratory animal welfare among undergraduate and graduate students of veterinary medicine in southeastern China. The investigation mainly focused on evaluating students' levels of knowledge regarding animal welfare, their proficiency with experimental techniques, and their opinions on approaches to teaching animal welfare. Additionally, we analyzed existing educational practices and identified challenges related to the welfare of and ethical issues surrounding laboratory animals in colleges and universities. Based on our survey results, we aim to optimize education and training on experimental animal ethics for veterinary students. This will enhance the quality of teaching about animal welfare and subsequently promote the professional competence of future veterinarians.

2 Materials and methods

A self-designed questionnaire was used to evaluate the current opinions of university students on laboratory animal welfare. Different kinds of questions were included: responses were given in multiple-choice form (including questions with single-answer choices and with single- or multiple-answer choices) or on a Likert scale. The questionnaire consisted of similar content to that of other laboratory animal studies in China and abroad (17–19), ensuring comprehensive coverage. The survey had no time restrictions, but respondents were expected to complete it within 10 min. The questionnaire was handed to the students by the head teacher of the class after a class meeting. Anonymous participation was ensured, and only undergraduate (senior and junior) and graduate students of veterinary medicine, who have direct contact with experimental animals, were targeted. No specific incentives were offered.

The questionnaire was divided into four sections: (1) the respondent's understanding of concepts related to animal welfare and ethics; (2) the current status of education on laboratory animal ethics; (3) attitude toward laboratory animals and mastery of animal experimentation skills; and (4) opinions on teaching and awareness of topics surrounding the welfare of and ethics issues relating to laboratory animals.

All data were imported into a Microsoft Excel spreadsheet (Microsoft Corporation, Redmond, WA). A total of 300 surveys were collected, of which 298 surveys were included in the analysis, comprising responses from 150 undergraduates and 148 graduate students. The response rate was 99.3%. Descriptive statistics for all variables of interest were tabulated in Excel. One of the authors individually screened each survey to calculate the percentage (%) of questions answered.

This study received approval from the Ethics Committee of Scientific Research at Fujian Agricultural and Forestry University. Respondent information has been anonymized to ensure confidentiality.

3 Results

3.1 Awareness of animal welfare and ethics

Table 1 presents a summary of the responses regarding student awareness of animal welfare. Among undergraduate students, 86.7% were aware of the concept of animal welfare and ethics, while

TABLE 1 Level of understanding of concepts relating to animal welfare and ethics.

Question	Response	No. of respondents (% of survey sample)	
		Undergraduate students	Graduate students
Are you familiar with the concept of animal welfare and ethics?	Yes	130 (86.7%)	108 (72.9%)
	No	20 (13.3%)	40 (27.1%)
Do you know of the concepts of the "3Rs principle" and the "Five Freedoms" in animal welfare?	Yes, know well	39 (26.0%)	17 (11.5%)
	Yes, but don't know the details	62 (41.3%)	58 (39.2%)
	No	49 (32.6%)	73 (48.7%)

among graduate students, this proportion was 72.9%. However, only 26.0% of undergraduates and 11.5% of graduates were familiar with the "Five Freedoms" and "3Rs principle" in animal welfare. It was observed that many undergraduate students had heard of these concepts but were unfamiliar with their specific content. Moreover, 32.6% of undergraduates and 48.7% of graduates reported having no prior knowledge of animal welfare, indicating a general lack of awareness among students regarding animal welfare and ethics.

3.2 Status of ethics education received on the utilization of experimental animals

Among the respondents, a majority of undergraduates (85.3%) and graduate students (87.9%) thought that they lacked knowledge of animal ethics in relation to laboratory animals. However, a considerable proportion of undergraduates (68.7%) and graduate students (60.1%) still expressed the intention of giving due consideration to the welfare of laboratory animals during animal experiments. Furthermore, most of the respondents believed that the teaching of laboratory animal ethics would be beneficial to their future careers. Only a small proportion of undergraduates (2%) and postgraduate students (4.7%) believed that such teaching would not be helpful to their future careers (Table 2). These findings suggest that majority of students demonstrated recognition of the relevant ethical issues and a willingness to participate in education on laboratory animal ethics.

3.3 Attitude toward experimental animals and mastery of skills in animal experimentation

Table 3 presents an overview of the responses to multiple survey questions relating to animal experiments. When dissecting animals or performing other experiments on injured animals, 14.7% of undergraduate students and 12.2% of graduate veterinary medicine students expressed adopting a similar mindset to that adopted when performing physical or chemical experiments. A majority of undergraduate students (61.3%) and graduates (75.0%) reported that although they did not show their emotions during animal experiments, they were emotionally affected. Furthermore, 24.0% of undergraduates and 12.8% of graduates expressed difficulty in performing experiments on injured animals. Regarding upholding of the "humanitarian spirit" during experimental procedures on animals, 19.3% of undergraduate students believed that they did not uphold this principle at all. Most of the students (66% of undergraduates and 81.8% of graduates) indicated that while they aimed to maintain the humanitarian spirit, their skills in experimental animal procedures did not meet the relevant standards. Only a small percentage of undergraduate students (6.0%) and postgraduates (10.1%) reported both upholding the humanitarian spirit and complying with technical specifications. The results of these two components of the survey demonstrate that most of the students prioritized the welfare of laboratory animals and strove to adhere to the guidelines for animal experiments. However, there appeared to be a general lack of proficiency in experimental techniques among current students, with only a small number lacking a humane approach.

3.4 Opinions on teaching and advocacy relating to laboratory animal welfare and ethics

Table 4 presents the findings from the section of the survey on teaching and advocacy relating to laboratory animal welfare and ethics. The results indicated that a significant majority of undergraduate (96.7%) and graduate (98.6%) students in China acknowledged their responsibility to advocate for and implement measures ensuring animal welfare. Moreover, a high proportion of students (specifically, 94.7% of undergraduates and 98.6% of graduates) believed that incorporating animal welfare knowledge and methods into the current teaching curriculum would be crucial. These results showed that most of the students accepted the provision of education on animal experiments and supported the ethical supervision of animal experiments. However, most of the students, particularly undergraduates (81.3%), were unaware of the existence of the Ethics and Animal Welfare Committee. Despite this, the majority of undergraduate (92.0%) and graduate (94.6%) students still expressed their support for the supervision of experimental animal welfare in teaching and research, highlighting the students' willingness to prioritize animal welfare and the need for further education and awareness regarding ethical considerations relating to laboratory animal experiments.

Table 5 presents the survey results indicating students' preferences and priorities regarding different teaching methods and content relating to laboratory animal welfare and ethics. Among

TABLE 2 Opinions on the status quo of education received on laboratory animal ethics.

Question	Response	No. of respondents (% of survey sample)	
		Undergraduate students	Graduate students
Do you think you lack knowledge of animal ethics in relation to laboratory animals?	Yes	128 (85.3%)	131 (87.9%)
	No	22 (14.7%)	17 (12.1%)
Will you pay more attention to the welfare of experimental animals in the process of conducting experiments?	Yes	103 (68.7%)	89 (60.1%)
	No	47 (31.3%)	59 (39.9%)
Do you think education on laboratory animal ethics will be helpful for your future career?	Yes	147 (98.0%)	141 (95.3%)
	No	3 (2.0%)	7 (4.7%)

TABLE 3 Attitudes toward experimental animals and mastery of experimental skills.

Question	Response	No. of respondents (% of survey sample)	
		Undergraduate students	Graduate students
How do you feel when you dissect animals or perform other experiments that cause injury to animals?	I adopt the same mindset as for physical or chemical experiments	22 (14.7%)	18 (12.2%)
	Although I don't show it, I still feel something	92 (61.3%)	111 (75.0%)
	It feels difficult to perform these experiments	36 (24.0%)	19 (12.8%)
Is humanitarianism maintained during animal experiments?	I feel nothing at all	29 (19.3%)	12 (8.1%)
	Yes, but the procedures are not standard	112 (74.7%)	121 (81.8%)
	Yes, the procedures are standard	9 (6.0%)	15 (10.1%)

undergraduate students, the highest proportion (83.3%) were willing to accept alternative teaching methods for experimental skills. This was followed by the degree of acceptance of teaching based on computer simulations (76.7%), specimens (67.3%), and videos (46%). Only a small percentage of students (2%) expressed unwillingness to accept any of these alternative methods. Among graduate students, the most widely preferred teaching method was model-based teaching, with 58.1% of respondents expressing this preference. This was followed by teaching based on specimens (45.3%), computer simulations (41.2%), and videos (36.5%). It is worth noting that a larger proportion of graduate students (26.4%) compared to undergraduates expressed unwillingness to accept alternative methods. Regarding curriculum content, both undergraduate and graduate students considered the practical application of laboratory animal welfare to be the most important area of knowledge within laboratory animal ethics, with this opinion expressed by 91.3% and 94.6% of respondents, respectively. This was followed by discussion of ethical issues relating to laboratory animals (regarded as important by 81.3% of undergraduates and 72.0% of graduates), study of the relevant laws and regulations (71.3% of undergraduates, 77.7% of graduates), and the differential treatment of different species (68.6% of undergraduates, 62.2% of graduates). Only a very small percentage of students (0.7% of undergraduates, 2% of graduates) stated that they did not consider any of this content to be important. Overall, the survey findings indicated the students' willingness to explore alternative teaching methods and their recognition of the importance of practical applications, ethics discussions, laws and regulations, and species-specific considerations in education on laboratory animal welfare and ethics.

4 Discussion

Animal experiments play a crucial role in learning and skill development among undergraduate and graduate students studying veterinary medicine. However, these experiments also raise concerns regarding the welfare of and ethics of the use of laboratory animals (20, 21). It is therefore essential for students to gain a comprehensive understanding of, and to actively incorporate, measures to ensure the welfare of these animals during their university education. Doing so not only fosters their sense of empathy and compassion but also cultivates their professional competence in this domain.

Veterinary students are expected to make a strong commitment to animal welfare and demonstrate a sense of responsibility in relation to the treatment of animals. Research has indicated that

TABLE 4 Opinions on teaching and advocacy relating to laboratory animal welfare.

Question	Response	No. of respondents (% of survey sample)	
		Undergraduate students	Graduate students
Do you consider yourself responsible for advocating for and implementing measures promoting animal welfare in our country?	Yes	145 (96.7%)	146 (98.6%)
	No	5 (3.3%)	2 (1.4%)
Under the current teaching curriculum, do you think it is necessary to teach knowledge and skills relating to animal welfare?	Yes	142 (94.7%)	146 (98.6%)
	No	8 (5.3%)	2 (1.4%)
Do you think that teaching or scientific research related to laboratory animals requires supervision by an animal welfare regulator?	Yes	138 (92.0%)	140 (94.6%)
	No	12 (8.0%)	8 (5.4%)
Are you aware of the existence of the Ethics and Animal Welfare Committee?	Yes	28 (18.7%)	45 (30.4%)
	No	122 (81.3%)	103 (69.6%)

TABLE 5 Opinions on teaching and advocacy relating to laboratory animal welfare (multiple-choice questions).

Question	Response	N. of respondents (% of survey sample)	
		Undergraduate students	Graduate students
Which of the following laboratory teaching alternatives would you accept or advocate for?	Teaching by model	125 (83.3%)	86 (58.1%)
	Specimen-based teaching	101 (67.3%)	67 (45.3%)
	Computer simulation teaching	115 (76.7%)	61 (41.2%)
	Video-based teaching	69 (46.0%)	54 (36.5%)
	Would not advocate for any of these alternatives	3 (2.0%)	39 (26.4%)
If you want to learn about laboratory animal ethics, which of the following do you think is the most important?	Discussion of ethical issues surrounding laboratory animals	122 (81.3%)	108 (72.0)%
	Practical application of the principles of laboratory animal welfare	137 (91.3%)	140 (94.6%)
	Differential treatment of different species	103 (68.6%)	92 (62.2%)
	Relevant laws and regulations	107 (71.3%)	115 (77.7%)
	No important content	1 (0.7%)	3 (2.0%)

veterinarians play a crucial role in identifying incidents of animal cruelty and domestic violence (13, 22–24). In the section of the survey on recognition of animal welfare and ethics, it was observed that while most respondents claimed to be familiar with these concepts, their knowledge of internationally recognized animal welfare standards was limited.

The 3Rs principle (Replacement, Reduction, and Refinement) provides ethical guidelines for the assessment and regulation of animal experimentation (25). This principle, which has been incorporated into guidelines and laws, ensures that animal experimentation meets both ethical and scientific criteria (26). The Five Freedoms (freedom from hunger and thirst; freedom from pain, injury, and disease; freedom from discomfort; freedom from fear and distress; and freedom to express normal behavior) established the five domains of animal welfare in the early 1990s and are now well recognized as highly influential in the animal welfare arena (27–29). The findings of this study indicate that a significant number of respondents were unfamiliar with the 3Rs principle and the Five Freedoms. This suggests that students generally lack a comprehensive understanding of animal welfare and ethics, highlighting the need for veterinary education programs to provide detailed instruction on these fundamental concepts, particularly for graduate students. In contrast, the results of a previous study with Italian students showed that respondents considered their own level of knowledge on the topic of animal welfare to be good (30).

Understanding the attitudes and perceptions of veterinary students in relation to animal welfare is fundamental in assessing the effectiveness and adequacy of their education (31, 32). The results of the current study indicate that students generally recognize the importance of education in laboratory animal ethics for their future careers and express a willingness to prioritize welfare issues during the development of experimental skills. In this study and in results presented by Pirrone et al. (13), it can be seen that the majority of students are open to receiving education in laboratory animal ethics, both to enhance their professional skills and to enable them to uphold humanitarian values.

It is important to note that while the majority of undergraduate and graduate students demonstrate compassion and care for experimental animals, there are still a small number of students who view them merely as tools for learning, adopting an apathetic attitude and lacking awareness of animal welfare and ethics. This highlights the need for further education and training to instill a stronger sense of empathy and ethical responsibility in these students.

Furthermore, the use of non-standard techniques and practices in laboratory animal experiments by some students is concerning. It is crucial for both undergraduate and graduate students to receive adequate training in experimental procedures in animals in order to minimize harm to animals and ensure the successful completion of experimental research. These issues are notable because these veterinary students represent future industry stakeholders who will play a role in addressing laboratory animal welfare issues and finding solutions to various welfare challenges in animal research (33, 34). One of the most common barriers to animal welfare mentioned in a previous survey is a perceived lack of researcher support to employ appropriate techniques (35). Therefore, both undergraduate and graduate students should receive better training in experimental procedures in animals; not only would this reduce animal suffering, but it would also provide them with a crucial foundation for performing successful experimental research (36, 37).

Veterinarians have a professional and ethical obligation to prioritize and promote animal welfare (38). As future practitioners in the veterinary industry, most veterinary students recognize and embrace this responsibility and express a strong sense of responsibility to advocate for animal welfare. They understand the importance of preventing harm to animals and protecting their welfare (39). To address these issues, various bodies such as Ethics and Animal Welfare Committees (AECs) and Animal Care and Use Committees (ACUCs) have been established in developed countries (40, 41). These committees evaluate research projects conducted by authorized institutions and provide reasoned opinions on them, weighing the potential human benefit against the harm caused to animals (40, 42). However, in China, the development of such bodies is still in progress, and there is room for improvement. This survey showed that, although most of the students were unaware of the existence of the Ethics and Animal Welfare Committee, they still recognized the importance of supervision. This indicates the pressing need to strengthen the publicization and awareness of these ethics committees in Chinese colleges and universities. Alternatively, it may be beneficial to establish subsidiary committees, such as a welfare supervision committee at the department or student level, to strengthen the supervision of animal welfare in daily teaching and scientific research activities. This would allow for better monitoring and implementation of animal welfare standards in research and education practices in China.

At present, there are various alternative methods of teaching that can be used in place of traditional animal experimentation, such as teaching based on models, computer simulations, specimens, or videos, among other methods (43, 44). Among these, model-based teaching, as the method that undergraduates and postgraduates are most receptive of, warrants support and promotion within university education. However, it is worth noting that a significant proportion of graduate students in this study expressed unwillingness to accept alternative teaching methods, which aligns with the findings of previous studies (45). Upon further investigation, it was found that these students believed that traditional teaching methods helped them to remember new knowledge and technological skills more easily. As a result, these graduate students suggested that a combination of traditional training methods and alternative approaches should be used to achieve the best learning results (46). In terms of the content of teaching materials, the practical application of laboratory animal welfare principles was considered by the students to be the most important component of knowledge of laboratory animal ethics. Additionally, most students deemed the discussion of ethical issues relating to laboratory animals and relevant laws and regulations to be important, and these topics should be included in future teaching curricula (47, 48).

The responses to the questionnaire highlighted the importance of education on laboratory animal ethics and revealed the overall views of veterinary students on laboratory animal welfare and ethics. The survey also identified a general lack of awareness among students regarding knowledge of laboratory animal ethics and relevant technology, laws, and regulations. This gap has been highlighted in other surveys as well, indicating the need for further training and awareness campaigns covering the scientific, legal, and ethical facets of laboratory animal research (30). It is apparent that the responsibilities of future veterinarians extend beyond the diagnosis, treatment, and prevention of animal diseases. They also have a crucial role to play as experts in and advocates for animal welfare and ethics. Therefore, both teachers and students should prioritize the implementation of the 3Rs principle in animal experimentation and acquire advanced technical skills and knowledge relating to laboratory animals. This is important not only for the personal growth and development of students but also for their ability to conduct high-quality scientific research and to progress in their future careers.

5 Conclusion

In conclusion, it is critical for veterinary students to be exposed to education in laboratory animal welfare throughout their undergraduate and graduate studies. Regardless of the specific course, any education related to animals should include the development of awareness of the importance of protecting the rights and welfare of laboratory animals. Universities should

prioritize strengthening formal education on topics such as animal medicine, animal surgery, and the technical skills necessary to perform procedures. The aim of these efforts is to cultivate appropriate professional attitudes and ethical practices and the necessary knowledge and skills to ensure safe and effective practices among veterinary students. By integrating laboratory animal welfare education into the overall learning process, we can better prepare future veterinarians to prioritize the wellbeing and ethical treatment of all animals in their care.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

This study was approved by Ethics Committee of Scientific Research of Fujian Agricultural and Forestry University, (Permit Number 111421143). All surveys were carried out according to the regulations and the respondent information is anonymized. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

SY: Writing — original draft. HL: Writing — original draft, Investigation. JL: Investigation, Writing — original draft. HC: Data curation, Writing — original draft. SL: Writing — review & editing. HD: Writing — review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This research was supported by the Undergraduate Education and Teaching Reform Research Program of Fujian Agriculture and Forestry University (111421143, 111423041) and the Graduate Education and Teaching Reform Research Program of Fujian Agriculture and Forestry University (712018270373, 712018270502).

Acknowledgments

We thank Kul Raj Rai of our laboratory for helpful assistance.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Veterinary Science

TYPE Review
PUBLISHED 16 February 2024
DOI 10.3389/fvets.2024.1366933

OPEN ACCESS

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RECEIVED 07 January 2024
ACCEPTED 01 February 2024
PUBLISHED 16 February 2024

CITATION
Whitham JC and Miller LJ (2024) Utilizing vocalizations to gain insight into the affective states of non-human mammals.
Front. Vet. Sci. 11:1366933.
doi: 10.3389/fvets.2024.1366933

Utilizing vocalizations to gain insight into the affective states of non-human mammals

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This review discusses how welfare scientists can examine vocalizations to gain insight into the affective states of individual animals. In recent years, researchers working in professionally managed settings have recognized the value of monitoring the types, rates, and acoustic structures of calls, which may reflect various aspects of welfare. Fortunately, recent technological advances in the field of bioacoustics allow for vocal activity to be recorded with microphones, hydrophones, and animal-attached devices (e.g., collars), as well as automated call recognition. We consider how vocal behavior can be used as an indicator of affective state, with particular interest in the valence of emotions. While most studies have investigated vocal activity produced in negative contexts (e.g., experiencing pain, social isolation, environmental disturbances), we highlight vocalizations that express positive affective states. For instance, some species produce vocalizations while foraging, playing, engaging in grooming, or interacting affiliatively with conspecifics. This review provides an overview of the evidence that exists for the construct validity of vocal indicators of affective state in non-human mammals. Furthermore, we discuss non-invasive methods that can be utilized to investigate vocal behavior, as well as potential limitations to this line of research. In the future, welfare scientists should attempt to identify reliable, valid species-specific calls that reflect emotional valence, which may be possible by adopting a dimensional approach. The dimensional approach considers both arousal and valence by comparing vocalizations emitted in negative and positive contexts. Ultimately, acoustic activity can be tracked continuously to detect shifts in welfare status or to evaluate the impact of animal transfers, introductions, and changes to the husbandry routine or environment. We encourage welfare scientists to expand their welfare monitoring toolkits by combining vocal activity with other behavioral measures and physiological biomarkers.

KEYWORDS

animal welfare, emotion, affective state, vocalization, bioacoustics

1 Introduction

Welfare scientists are continually searching for non-invasive, animal-based measures that can be tracked on a regular basis to provide insight into an individual's welfare status (1–3). Animal welfare is measured on a continuum from poor to good and considers an individual's mental, physical, and emotional or affective states (2). One way to gain insight into an individual's inner, affective state is to examine vocal behavior, which can reflect physical, behavioral, and psychological aspects of welfare (4–11). Affective states are emotional experiences that overlap on a spectrum, ranging from fleeting emotions—often triggered by

a specific event or object—to longer-term moods (12). Furthermore, it is important to note there are two core dimensions of affect—arousal (intensity) and valence (positive vs. negative). The term *vocalization* is defined here as the active generation of sounds by the vocal tract (pharynx, vocal, nasal and oral cavities, lips and nostrils) that express a distinctive inner state, occurring spontaneously or as the result of an external event (8; Table 1). While this review primarily focuses on vocalizations that are audible to humans, some species also emit ultrasonic or infrasonic calls that may be associated with particular affective states and relevant to welfare status. Vocalizations are valuable for examining the expression of emotions, as sound typically travels well around obstacles, carries long distances, and can change quickly depending on the situation (8, 10, 11, 13). While most studies on non-human animals have investigated the types, rates, and acoustic features of vocalizations emitted in negative contexts, acoustic activity can also serve as an indicator of positive, pleasurable affective states [e.g., (5, 9, 10, 14–18)]. Indeed, Fraser (15) argues that similar to how animals have evolved systems to signal hunger or distress, some species may have evolved to emit signals of positive affect. After all, positive vocalizations can serve a vital communicative function for social, group-living species, by promoting the formation of social bonds and cooperation [e.g., (11, 19–21)].

TABLE 1 Main acoustic parameters and terms discussed in the current article.

Acoustic parameter/term	Definition/Description
Amplitude	Level of energy in a vocalization. Involves the lungs and trachea.
Bandwidth	The difference between the highest and lowest frequency.
Duration	The length of a vocalization from start to finish. Involves the lungs and trachea.
Formants	Frequencies that correspond to the vocal tract's resonances. Involves the vocal tract (pharynx, vocal/nasal/oral cavities, lips, and nostrils).
Frequency modulation	Variability of the dominant frequency or F0 across the call.
Fundamental frequency (F0)	The lowest frequency in a vocalization. Involves the lungs, trachea, and larynx.
Phonation	The transformation of air flow into sound by vocal fold oscillation.
Spectral noise	Proportion of noise in the vocalization, where the harmonic structure is not clear or cannot be detected.
Vocalization	The active generation of sounds by the vocal tract (pharynx, vocal, nasal and oral cavities, lips and nostrils) that express a distinctive inner state, occurring spontaneously or as the result of an external event.
Vocalization rate	The number of calls that occur per time unit.

Adapted from Briefer (10) and Laurijs et al. (11).

This review focuses on the acoustic activity of non-human mammals. A vast amount of literature exists on human vocal behavior and will primarily be referenced here to gain better insight into the findings for non-human mammals. Across mammals, there are acoustic correlates of the core dimensions of affect—i.e. arousal and valence (10, 12). Overall, when looking across mammalian species, calls increase in the rate of production as arousal increases (10, 22). Affective state can also influence the acoustic features of vocalizations, including the call's duration, fundamental frequency (i.e., F0, or the lowest frequency of the vocalization), formants (i.e., frequency peaks in the spectrum), and amplitude (10, 22, 23). As arousal increases, the acoustic structure of vocalizations changes in a predictable way across mammalian species, with calls increasing in amplitude and frequency (both F0 and formant-related frequencies) and F0 becoming more variable (10, 23). In other words, as arousal increases, calls are emitted at faster rates and become louder, longer, and harsher (10). However, when considering valence, changes are less consistent across species. In general, a shift in valence tends to be associated with a change in call type (e.g., laughing to crying in humans; whinnies to squeals in horses, *Equus caballus*) (10, 24). Furthermore, calls emitted in positive situations are typically shorter in duration than those that occur in negative contexts (10, 18, 20, 23, 25–31). When the same call type is emitted in both negative and positive contexts, those that occur in positive contexts tend to be shorter in duration but may shift higher or lower in terms of fundamental frequency, depending on the species and/or call of interest [e.g., (10, 18, 28, 32–36)].

This article examines how vocal behavior can provide insight into the affective states of non-human mammals. Specifically, we will:

1) Provide a brief overview of vocal production in non-human mammals.
2) Investigate the construct validity of vocal indicators of affect, with a focus on measures of emotional valence. To review the evidence that vocalizations can be utilized as valid indicators of affective state, we will consider whether vocalizations: (a) reliably vary when individuals experience conditions that are aversive or preferred, (b) reliably vary when individuals experience conditions known to reduce or enhance fitness or survival, (c) are associated with previously validated welfare indicators, and (d) reliably vary when individuals undergo brain stimulation or receive drugs that modulate affect (37). We acknowledge that, at this time, vocalizations are more likely to provide insight into short-term affective states rather than longer-lasting moods.
3) Review methodological considerations and limitations for welfare scientists planning to examine the relationship between acoustic activity and affect.
4) Discuss how the study of vocal behavior can be applied to monitoring the affective states and welfare of animals living under professional care. While the value of tracking vocalizations has been recognized by some welfare scientists working with zoo/aquarium, companion, laboratory, and farm animals, acoustic activity generally has been underutilized in welfare research. We discuss: (a) identifying potential vocalizations of interest by considering a species' natural history, (b) validating vocal indicators of affect, and (c) incorporating these indicators into welfare monitoring schemes.

2 Vocal production

The ability to emit vocalizations relies on the presence of a vocal tract, which in mammals, is characterized by specialized features of both the tracheal tract and pharyngeal cavities (8, 10, 11). According to the source-filter theory (38, 39), the vocalizations emitted by mammals are produced by vibrations of the vocal folds in the larynx (source) and then filtered in the vocal tract (filter). The source determines the fundamental frequency of the call. This aspect of vocal production is influenced by both respiration and phonation (i.e., the transformation of air flow into sound by vocal fold oscillation), thereby involving the lungs and trachea (38). The sound waves generated by the larynx are then filtered by the supralaryngeal vocal tract (filter) (10, 39, 40). The filtering mechanism of the vocal tract—which involves the pharynx, vocal/nasal/oral cavities, lips, and nostrils—shapes the energy distribution of the call and creates the formants by amplifying some frequencies and dampening others (38).

There is evidence that filter-related parameters can provide information about valence (10, 23, 41–44). Indeed, research on humans has shown that filter-related cues vary when comparing emotions that differ in valence but are characterized by similar levels of arousal [e.g., (41, 43, 45, 46)]. As described below, some studies on non-human mammals have examined formants, which may be the key to investigating emotional valence in the future (10, 23, 26, 33, 47). Briefer (10) argues, "it is crucial to measure a large set of parameters including formant frequencies, using the source–filter framework, in order to obtain emotion-specific vocal profiles" (p. 5).

3 Evidence of construct validity

There is mounting evidence that vocalizations can be utilized as valid, non-invasive indicators of affective state for non-human mammals. We do not provide a thorough review of the human vocal expression literature here, though studies on human subjects do allow researchers to examine how vocalizations can reliably map onto self-reported affective states (48). For more details on construct validation of vocal indicators of emotions, as well as sensitivity and specificity issues, please see Villain and Briefer (49).

3.1 Vocalizations emitted in aversive or preferred contexts

3.1.1 Vocalizations emitted in aversive contexts

Vocalizations emitted in situations that are assumed to be aversive may be indicative of negative affect. Social isolation or separation, which at the very least are considered to be unpleasant for socially-living animals, are associated with changes in acoustic activity for some species [e.g., (5, 50–54)]. In general, mammalian young vocalize frequently when separated from their mother and/or litter-mates [e.g., (5, 50)]. Moreover, numerous studies have demonstrated that a wide range of species emit isolation calls that vary in acoustic structure in relation to various factors (e.g., olfactory, tactile, thermal, early experience, postnatal maternal separation) [e.g., (5, 51–54)]. For instance, Weary et al. (5) discovered that male suckling piglets (*Sus scrofa domesticus*) call repeatedly when isolated from their mother and litter-mates, with those isolated in a cool enclosure vocalizing more often and producing longer, higher frequency calls than those isolated in a warmer enclosure. While these vocalizations appear to be an honest, reliable indicator of need, it can also be assumed that the piglets are experiencing a negative emotional state. Similarly, when being restrained by females who are not their mothers, infant rhesus macaques (*Macaca mulatta*) produce noisy screams, with riskier, severe situations (i.e., longer periods of restraint) being associated with a greater number of calls (55). Finally, in a study of Weddell seal (*Leptonychotes weddellii*) pups, the calls produced by lone pups and those reuniting with their mothers were characterized by longer durations, higher rates of emission, and higher fundamental frequencies than calls emitted during mother-pup contact periods (56). However, these variations in vocal parameters between contexts seem most consistent with the pups' expression of arousal (10). This is likely the case for many mother-offspring separation studies.

In addition, changes in vocal activity have been reported for adult mammals separated or isolated from conspecifics and are generally considered to be indicative of distress (57–59). In fact, the intensity and frequency of calls may even reflect the strength of the bond between two individuals (58). A study on male cheetah (*Acinonyx jubatus*) pairs found that subjects vocalized at higher rates when separated than during reunions and that sibling pairs vocalized at significantly higher rates than non-siblings (58). Chirps, which exhibited the most individual distinctiveness, were the most common calls produced during separations and only stutters were recorded during reunions. The authors note that in carnivores, short, high-frequency vocalizations with abrupt onset (e.g., the chirp of cheetahs) reflect fear or distress, while low-frequency pulsed and low-amplitude modulated vocalizations (e.g., the stutter of cheetahs) are emitted in affiliative contexts (58; see also 60, 61). Finally, Siebert et al. (62) reported that dwarf goats (*Capra hircus*) emit fewer high bleats but more low bleats when completely isolated, as compared to when they are partially isolated. The authors argue that low bleats may serve a self-calming mechanism and reflect a form of auto-communication. In sum, call type, rate, and even structure may vary when social mammals face separation or isolation from conspecifics.

Alterations in vocal behavior and the acoustic features of calls may also be associated with environmental disturbances. The vocal behavior of farmed silver foxes (*Vulpes vulpes*), who are generally fearful of humans, was impacted by changes in animal-human distance during a human approach test (63). Specifically, the foxes spent an increased proportion of time vocalizing and vocalized at higher frequencies as humans approached. Gogoleva et al. (63) argue that for this species, these variables may, "represent reliable indicators of short-term welfare problems" (p. 8). Alternatively, Castellote and Fossa (64) discovered that the overall vocalization rate (i.e., rate of all vocalization types combined) of beluga whales (*Delphinapterus leucas*) decreased drastically following transportation to new facilities and remained low for four weeks. Notably, food intake was not significantly impacted by the move, and while the whales did display some negative behaviors (e.g., inattentiveness during feeding sessions, low interest/motivation to interact with trainers), these behaviors were not reported by trainers after day eight. Willingness to participate (WtP) in training sessions is an important measure, as a study on bottlenose dolphins (*Tursiops truncatus*) found that WtP was lower in the days leading up to a veterinary diagnosis of a decrease in health state (65). Finally, it should be noted that the overall vocalization rate of the beluga whales studied by Castellote and Fossa (64) also decreased following the introduction of harbour seals (*Phoca vitulina*) and remained low for two weeks. This introduction to the seals occurred

approximately four months after the move to the new facility. It is possible that that the acoustic behavior of beluga whales, and specifically the overall production of vocalizations, may be a better welfare indicator than other behaviors (64).

Many studies have investigated the calls emitted by individuals in the context of aggression and social tension. Morton's (66) "motivational-structural rules" proposed that animals typically emit low-frequency (i.e., low pitch), wide-bandwidth (i.e., noisy) calls in hostile, agonistic contexts and high-frequency (i.e., high pitch), narrow-bandwidth (i.e., tonal) sounds when expressing fear or interacting in a friendly or appeasing manner. Since then, several studies have examined and largely supported these assumptions, though the findings are more consistent for the aggressive contexts [e.g., (67–73)]. In general, vocalizations that occur during agonistic interactions are characterized by long durations, low frequencies, minimal frequency modulations, and wide frequency ranges (reviewed by 10). Some inconsistencies of Morton's theory may be explained by the fact that both aggression and fear are negatively valenced, while friendly, affiliative behaviors are positively valenced (12, 61). As a result, variations of Morton's theory have been proposed by other researchers [e.g., (61)].

3.1.2 Vocalizations emitted in preferred/positive contexts

Although it is less common to investigate calls emitted in positive contexts, numerous welfare researchers argue that it is crucial to examine vocalizations that reflect positive affect, as the lack of negative indicators does not imply that an animal is experiencing pleasure or good welfare (3, 9, 15, 74). Indeed, Fraser (15) highlights the value of investigating the noises produced when "all's well." Some species' vocal repertoires include calls that are associated with positive affect. For instance, like humans, orangutans (*Pongo pygmaeus*), bonobos (*Pan paniscus*), chimpanzees (*Pan troglodytes*), gorillas (*Gorilla gorilla*) and siamangs (*Symphalangus syndactylus*) "laugh" when tickled (17). Similarly, Panksepp and Burgdorf (14) suggest that the 50 kHz ultrasonic chirps produced by adolescent rats (*Rattus norvegicus*) while playing and being tickled by experimenters are reminiscent of primitive human laughter (see also 75). In fact, Burgdorf et al. (76) suggest that these emotional vocalizations can serve as "self-report" measures when rats experience positive affective states (20, 75). As noted above, Briefer's (10) review found that vocalizations emitted in positive contexts tend to be shorter in duration than those that occur in negative contexts, but can vary greatly in fundamental frequency. Indeed, as opposed to the high-frequency ultrasonic vocalizations emitted by rats, some species emit low-frequency calls in positive contexts, including the murmuring of ruminating cows (*Bos taurus taurus*) (77), the coos of infant rhesus macaques (55), and the purrs of gray mouse lemurs (*Microcebus murinus*) receiving grooming from an experimenter (78). Purring and purring-like vocalizations have been reported in the context of relaxed, affiliative interactions (e.g., huddling, mutual grooming, friendly approach) for a wide variety of mammals, including ring-tailed lemurs (*Lemur catta*), northern tree shrews (*Tupaia belangeri*), raccoons (*Procyon lotor*), and various felid species (reviewed by 79). Peters (79) suggests that, in general, purring-like vocalizations, "denote that the vocalizing individual is 'feeling well', 'comfortable' or 'content'…" (p. 264). However, it should be noted that purring-like vocalizations have been also reported in animals experiencing pain or distress and may therefore reflect self-soothing, appeasement, or aversive emotional states (79). Ultimately, welfare researchers should specifically attempt to identify vocalizations that are associated with positive events, experiences, and states for the species of interest (see Section 5 for further discussion).

3.1.3 Identifying indicators of valence

To identify indicators of valence, some researchers analyze vocalizations produced in *both* positive and negative situations, and in some cases, attempt to control for arousal. This "dimensional approach" proposes that each emotion can be mapped by simultaneously considering both valence and arousal (12, see also 48, 80; Figure 1). For example, vocal indicators of being "relaxed" will be reliably associated with positive valence and low arousal, while indicators of being "anxious" will be associated with negative valence and high arousal. Ultimately, positive affective states will encourage animals to approach stimuli that can promote fitness, while negative affective states trigger avoidance of potentially dangerous stimuli (12).

Most studies that have set out to identify indicators of valence have been conducted on domesticated animals and related species. Briefer et al. (34) identified non-invasive, reliable indicators of both arousal and valence in goats by exposing subjects to four situations (control, anticipating a food reward, food-related frustration, and isolation), for which arousal level could be assessed by measuring heart rate. In positive situations, the goats emitted vocalizations with a lower fundamental frequency range and smaller frequency modulations. In another study that used behavioral and physiological measures to control for arousal, closed-mouth grunts produced by adult pigs during a positive situation (access to food/toys while paired with a conspecific) varied from those emitted during a negative situation (social isolation) in various ways, including shorter durations, differences in formant-related parameters (see below), and lower fundamental frequencies (23). Similar results were found for wild boars (*Sus scrofa*), with subjects emitting shorter, lower frequency

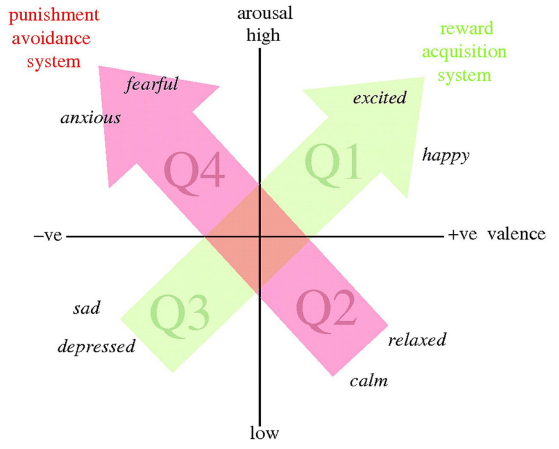

FIGURE 1
Core affect represented in two-dimensional space. Words in italics indicate possible locations of specific reported affective states (including discrete/basic emotions). Positive affective states are in quadrants Q1 and Q2, and negative states in quadrants Q3 and Q4. Arrows indicate putative biobehavioural systems associated with reward acquisition and the Q3–Q1 axis of core affect (green), and punishment avoidance and the Q2–Q4 axis of core affect (red). Adapted from Russell [e.g., (78)] and Panksepp [e.g., (48)]. Reproduced from Mendl et al. (12).

vocalizations in positive situations (food reward or affiliative contexts) than in negative situations (agonistic encounters), when controlling for arousal (30). In a study of horse whinnies, those produced in a positive context (reunion with group members) were found to be shorter in duration than whinnies emitted in a negative context (separation from group members) (28). Furthermore, two fundamental frequencies, F0 (the lower fundamental frequency) and G0 (the higher fundamental frequency), were discovered, with the latter encoding valence by being lower in positive situations. Interestingly, while whinny duration and G0 frequency were not a reliable indicator of valence in Przewalski's horses (*Equus przewalskii*): (1) positive and negative contexts were associated with particular call types and (2) acoustic structure varied according to valence (81). Overall, however, there is evidence that vocalizations emitted in positive contexts are generally shorter in duration with lower fundamental frequencies (10, 23).

The findings for domestic dogs (*Canis familiaris*) break from the pattern of calls having lower fundamental frequencies when emitted in positive versus negative contexts. For instance, in a study of dog barks, those produced in the context of play (and isolation) had a different acoustic structure—shorter inter-call intervals, shorter durations, higher frequencies, and more pitch and amplitude modulation —than barks emitted during "a disturbance situation" (stranger ringing the doorbell) (82). Similarly, Faragó et al. (26) reported that growls were characterized by shorter durations and higher fundamental frequencies in the context of play than in negative situations (exposure to a threatening stranger, food guarding) (26). On the contrary, a separate study of dog growls found that while growls produced in the context of play (playing with owner) were shorter than those emitted during the context of aggression (an approaching stranger), they did not vary in fundamental or formant frequencies (18). As noted earlier, when comparing the same call type across negative and positive situations for a particular species, the vocalizations produced in positive contexts may shift to a higher frequency (10, 23).

It is less common for studies investigating emotional valence to be conducted on non-domesticated species. In African elephants (*Loxodonta africana*), the rumbles produced by low-ranking females in calm social contexts differed from those emitted while interacting with dominant elephants, with the former having lower and less variable fundamental frequencies, as well as lower amplitudes and shorter durations (32). In a follow-up study, Soltis et al. (33) attempted to take a dimensional approach and control for arousal by examining rumbles in the following contexts: high intensity/negative social context (dominance interactions), high intensity/positive social context (affiliative interactions), and a low intensity/neutral social context. The authors found that females produced calls with longer durations, as well as higher and more variable fundamental frequencies and amplitudes, when comparing the negative context to the neutral context. However, rumbles emitted in the positive context were similar in duration to the negative context, and when considering most of the acoustic features (e.g., F0 range, amplitude range, max amplitude), the findings were intermediate between the neutral and negative contexts. Soltis et al. (33) concluded that the results were most consistent with rumbles reflecting affect intensity (regardless of valence), with the acoustic responses in the positive context signaling an intermediate level of arousal. However, they suggested that the combination of acoustic features may create a "vocal signature" of valence. After all, while the features that increase in both positive and negative contexts may reflect arousal, those that only increase in the negative context may reflect valence. A study of farmed spotted paca (*Cuniculus paca*) found that certain acoustic parameters of snorts, barks, and roars varied according to the valence of situation, and that snorts were more likely to be emitted in a negative situation (enclosure cleaning) than a positive situation (feeding time) (83). Finally, in a study of bonobo peep vocalizations, the acoustic structure of peeps produced during positive situations (feeding) could not be distinguished from those emitted during neutral situations (travel, rest) (35). However, peeps produced in negative situations (agonism and alarm) were characterized by shorter durations and higher mean fundamental frequencies.

Finally, just as research on humans has shown that formants may vary with valence, formant-related parameters may be valuable to the study of non-human animal emotions (10). Relatively few studies have examined formant-related features, and so far, the results are inconsistent. In Briefer et al's. (23) study of pigs' closed-mouth grunts, calls produced in the positive situation (access to food/toys while paired with a conspecific) were characterized by higher formants and a smaller range of the third formant than grunts emitted in the negative situation (social isolation). A study of feral cat (*Felis catus*) meows reported that the first formant of calls was higher in a positive context (affiliation) than a negative context (agonism) (47). Alternatively, for African elephants, rumbles produced in a negative social context (dominance interactions) had higher first formants than those emitted in a neutral context (33). For dogs, growls emitted in a positive context (play) were characterized by lower formant dispersion—which is suggestive of lower formants—than those produced in a negative context (exposure to a threatening stranger, food guarding) (26). As noted above, however, Taylor et al. (18) did not find differences in formant-related parameters when comparing dog growls produced in positive and negative contexts (play vs. aggression). Other studies described in this section, including the studies of horse whinnies (28) and goat calls (34), reported no significant effects of valence on formants. Further research is needed, as results may be influenced by the use of different species, contexts (e.g., play vs. affiliation for positive contexts, isolation vs. agonism for negative contexts), arousal levels, etc. (23).

3.2 Vocalizations emitted in situations that reduce or enhance fitness or survival

Numerous studies have investigated the calls emitted by animals facing situations that have the potential to reduce fitness or survival. There is evidence that vocalizations can be honest indicators of pain, which is assumed to be associated with negative affect and poor welfare [e.g., (84, 85)]. In general, animals experiencing severe pain emit calls as a high rate (84–87). For example, several studies have found that male piglets undergoing castration produce calls at high rates, with these vocalizations generally having high frequencies, amplitudes, and durations (85, 86, 88, 89). Furthermore, the calls emitted by piglets experiencing pain distress can be distinguished from vocalizations produced during other types of distress (i.e., cold and hunger) (90).

An extensive amount of research has been conducted on calls produced in fear-inducing situations, such as the alarm calls emitted

in the presence of predators (91, 92). It is important to note that while some species emit alarm calls frequently when facing predators or other potential threats, others may become less vocal or even silent (92, 93). For example, when both free-ranging and professionally-managed beluga whales (*Delphinapterus leucas*) are exposed to predators or noise disturbances (e.g., killer whales, boat engines), their acoustic activity may decrease or cease completely (64, 94–96). Similar results have been found for free-ranging narwhals (*Monodon monoceros*), suggesting that it may be adaptive for marine mammals to reduce or eliminate vocal activity when encountering potential threats (97). Indeed, it can be advantageous for frightened, startled, or threatened individuals to avoid detection in the presence of predators by remaining silent (64, 94). It is vital to investigate the evolution and natural history of specific vocalizations, as well as whether the call may have co-evolved with other behaviors (e.g., hiding vs. freezing) (4, 98). Section 5 further discusses the importance of understanding the natural history of a species' vocal repertoire.

3.3 Vocalizations associated with previously validated welfare indicators

Vocalizations produced in both negative and positive contexts are often temporally associated with previously validated physiological or behavioral welfare indicators (reviewed by 10). While researchers assessing the arousal and valence of calls should attempt to incorporate physiological indicators, relatively few studies have integrated biomarkers such as heart rate, respiration, adrenaline, or cortisol/corticosterone (50, 59, 99, 100; reviewed by 10). Overall, however, most evidence seems to point to physiological indicators being associated with indicators of emotional arousal (e.g., 34, 99; reviewed by 10).

Some studies have examined how vocal behavior is associated with cardiac activity or respiration rates. In Briefer et al's. (34) study of goat vocalizations, it was discovered that heart rate variability was not influenced by valence but was impacted by arousal, with high arousal situations being associated with lower heart rate variability and higher respiration rates (see also 101). The authors noted that they did not identify a good physiological indicator of valence. A similar conclusion was drawn for gilts participating in a standard human approach test. Specifically, gilts that squealed more not only displayed more locomotor behavior and interacted more with humans, they also had higher mean heart rates and lower heart rate rise in response to human touch (99). The authors argued that these findings were indicative of higher arousal levels.

Other researchers have investigated the relationship between vocal behavior and hypothalamic–pituitary–adrenal (HPA) axis activity. Boinski et al. (102) discovered that the group mean of terrestrial predator alarms (TPA) for singly housed adult male brown capuchins (*Cebus apella*) was positively correlated with mean group levels of fecal cortisol, as well as abnormal behaviors. Furthermore, it should be noted that those housed in an enriched environment (i.e., cages with toys and foraging boxes) had a lower mean TPA rate in response to humans than the control group (i.e., cages with only a plastic chain). As a result, the authors suggested that individuals housed under low enrichment conditions were more "stressed" and reactive and that TPAs can serve as a "first-line" indicator of welfare. In an experimental study that involved separating adult pigs from groupmates, it was determined that increasing rates of squeal-grunts were positively associated with plasma levels of adrenaline, while rates of grunts were inversely associated with cortisol levels (103). However, it should be noted that within these call types, acoustic parameters were not significantly correlated with either hormone. While laboratory-housed adult marmosets (*Callithrix jacchus*) produced high levels of phee calls when separated from groupmates and placed in a novel environment for 20 min, there was no association between the number of calls emitted and cortisol levels (59). The results from infant separation studies are also mixed. For instance, in a 2-wk maternal separation study involving infant bonnet macaques (*Macaca radiate*) and pigtail macaques (*Macaca nemestrina*), mean plasma free and total cortisol were positively associated with distress vocalizations and slouching and negatively associated with play during the first week of separation (104). However, in a study that examined the separation calls of infant squirrel monkeys (*Saimiri sciureus*) over a 24-h period, the intensity of calling was not predictive of cortisol levels (105). Clearly, such studies vary greatly in terms of the species of interest, age of the subjects, and methodology, including the length of the separation (hours vs. weeks).

If possible, researchers should attempt to combine measures of heart rate, HPA activity, and behavior. In a study that examined the responses of ewes (*Ovis aries*) separated from their lambs, ewes exhibited a significant increase in activity, vigilance, bleats, heart rate, and cortisol levels (100). Furthermore, these behavioral and physiological responses were correlated with changes in the ewes' voice characteristics, including an increase in total duration, energy, and fundamental frequency. The authors argue that these behavioral, physiological, and acoustic changes reflect negative emotional states and that certain bleat characteristics may serve as markers of distress.

3.4 Insights from brain stimulation studies and pharmacological research

Numerous studies have investigated the neural substrates underlying vocal behavior. The amygdala, which is involved in the expression of both negative and positive emotions in mammals, plays a role in vocal production (106; reviewed by 11). Furthermore, there is evidence that the various call types comprising a species' vocal repertoire are generated via specific pathways that begin in the amygdala (11, 106, 107). Indeed, Jürgens (108) was able to induce vocalizations in squirrel monkeys by electrically stimulating the anterior cingulate cortex, which receives input from the amygdaloid complex. The amygdaloid complex not only mediates certain emotions (e.g., fear, anxiety) but also regulates the HPA axis (109–111). Studies have shown that different circuitries are involved with calls linked to negative states versus positive states (20, 112). For instance, the two types of acoustically distinct ultrasonic calls produced by rats—the 22 kHz vocalizations produced in negative contexts and the 50 kHz calls produced in positive contexts—are linked to the neural substrates associated with the generation of negative and positive states (20, 113). Specifically, electrical activation of the mesolimbic cholinergic system induces a negative emotional state and the production of 22 kHz calls, while the activation of the mesolimbic dopaminergic system induces a positive emotional state and 50 kHz calls (see also 114, 115). Briefer (10) notes that specific brain circuits responsible for emotions have been linked to particular vocalizations in other species. If specific vocalizations can be induced (or inhibited) by stimulating (or

lesioning) particular parts of the brain, and if those parts of the brain mediate certain affective states, evidence exists for construct validity.

Vocalizations can also be induced (or inhibited) by administering drugs that impact brain circuits associated with emotional states [e.g., (116–118)]. In pigs, an increased vocalization rate and activity can be induced by centrally injecting subjects with anxiogenic peptides, such as corticotropin releasing hormone (117, 119). Alternatively, when female rhesus macaques were administered metyrapone to suppress cortisol production, subjects emitted significantly fewer alarm calls in response to their infants being threatened, as compared to controls (120). A series of studies reported that the 50 kHz ultrasonic vocalizations of rats increased in response to the administration of euphorigenic drugs, while sickness-inducing doses of lithium chloride decreased the number of these calls [e.g., (115, 121)]. While these invasive studies are not recommended for species living in certain settings (e.g., zoos, aquariums, shelters, and sanctuaries), and there are obvious ethical concerns, this line of research has informed studies of vocal behavior by highlighting the links between specific brain circuits, affective states, and vocalizations.

4 Methodological considerations and limitations

For those planning to integrate measures of vocal behavior into studies aimed at assessing the affective states of individual animals, several methodological considerations and limitations must be addressed. While not always realistic for animals living in certain professionally managed settings (e.g., zoos, wildlife sanctuaries), the most informative studies will incorporate: (1) spectrographic analyses to identify links between specific acoustic features and particular affective states and (2) experiments that adopt a dimensional approach (i.e., that examine vocal behavior in both positive and negative situations of similar arousal) (12). Indeed, when assessing and monitoring affect, the goal is to identify indicators of valence—not just arousal—by pinpointing which acoustic features are associated with negative versus positive contexts.

In some cases, it may only be feasible to examine vocalization rates. Depending on the species of interest, tracking the rate of combined vocalizations or of specific call types may provide useful information. Even for researchers who are unable to purchase recording equipment and can only conduct behavioral observations, it is possible to establish baseline vocalization rates for individual animals and to track this measure across various contexts. As described above, some species become less vocal or even silent when facing threats or environmental disturbances [e.g., (64)]. Animals experiencing severe pain, extreme lethargy, or learned helplessness—states likely associated with negative affect—may also remain silent. Furthermore, it is important to recognize that individuals of the same species may vary in terms of how vocal they are in particular contexts, which may be due to differences in genetics, temperament, and early experiences [e.g., (122–124)]. Finally, it is crucial to remember that some species produce ultrasonic or infrasonic vocalizations, which would be missed if not recorded with the proper equipment calibrated to detect the appropriate frequency range (125).

Fortunately, researchers and animal management staff who have the means to purchase recording equipment and software are able to take advantage of recent technological advances in the field of bioacoustics. Modern acoustic monitoring systems make it possible for researchers to continuously track individuals under a variety of circumstances (e.g., in the dark, underwater, after-hours), even for group-housed or nocturnal animals (126, 127). Before initiating a study, a considerable amount of effort must be invested in weighing options for recording and analyzing calls. Vocalizations can be recorded by introducing hydrophones or microphones to enclosures or by utilizing animal-attached devices (e.g., collars) (74). Clearly, characteristics of the species of interest (e.g., size, physical attributes, ability of the wearer/conspecifics to manipulate the device) will influence the type of recording device that is chosen. If animal-attached devices are not feasible, the researcher will have to determine whether to employ directional or omnidirectional microphones. Certain settings are associated with particular challenges when utilizing recording devices. For example, in zoos, visitors contribute noise to the environment, and certain surfaces (e.g., windows, glass panels) reflect sound or even mask calls (128). For enclosures with a water feature, the air-water interface can reflect and reverberate sounds (128). For species that spend most or all of their time underwater, life support systems may produce additional noise that may not only interfere with recordings but also influence vocal behavior (62). Finally, low frequencies, which can travel longer distances and are less likely to be impacted by dense vegetation, are more likely to be captured by microphones (128–130). As a result, careful consideration must be given to the placement of microphones in the enclosure. Schneider and Dierkes (128) recommend taking the height of the enclosure into account and localizing in three dimensions, though this can be difficult if the enclosure is uneven in height. These researchers advise using least four microphones for two-dimensional localization of a vocalization but caution that even more are necessary in large enclosures.

Recent advances in bioacoustics software allow for continuous monitoring, automatic detection of calls, and real-time sound analyses. For instance, Schneider and Dierkes (128) tested the LASER sound localization software, which can accurately estimate the position of the animal that is vocalizing, thereby allowing the call to be assigned to the correct subject. Even when considering otters, which move quickly and closely together in an aquatic environment, 78% of the calls could be assigned to the correct caller. Similarly, the National Marine Mammal Foundation's Welfare Acoustic Monitoring System (WAMS) employs hydrophones to continuously capture, count, and localize vocalizations, as well as specialized software that automatically compares the current data to historical output/baseline data. In fact, this real-time alert system includes an alarm module that triggers an email alert (complete with a call count, screenshot of the spectrogram, and localization information) if the call rate surpasses the user-defined threshold (127, 131). For farm animals, Briefer et al. (29) demonstrated that an automated recognition system allowed for real-time discrimination of valence, as well as the context of call production. The researchers assessed two methods for call classification in this study: (1) an image classification neural network (i.e., a machine learning model that can recognize patterns in images) based on spectrograms of calls and (2) a permuted discriminant functional analysis (i.e., a multivariate statistical method used in bioacoustics research to distinguish calls) based on selected vocal parameters—with the former having higher classification accuracy. The authors concluded that this automated emotion monitoring tool can ultimately be used to track welfare on farms. A thorough

discussion of automated acoustic monitoring, advanced computational audio analysis methods, and spectrographic analyses is beyond the scope of this paper and can be reviewed elsewhere (8, 40, 126).

5 Discussion

For researchers interested in assessing or monitoring the affective states of individual animals, integrating measures of vocal behavior can be extremely valuable. When initiating a study to investigate associations between acoustic activity and affect, the first step is to identify potential calls of interest by examining the natural history of the species' vocal repertoire (4). This can be accomplished by conducting preliminary observations and reviewing the literature (4, 64). Specifically, the researcher can better understand the role particular vocalizations play in the species' behavioral repertoire by examining the call from a developmental and evolutionary perspective (4). For example, a given vocalization may only be produced by specific age/sex classes or may have co-evolved and be temporally associated with other behaviors (98). While reviewing the literature, the researcher should determine whether there is evidence that the species of interest emits ultrasonic or infrasonic vocalizations, which cannot be perceived by humans. If feasible, the researcher can collect recordings from individuals of various age/sex classes and conduct spectrographic analyses to identify all vocalization types. Ultimately, studies may be limited to investigating vocalizations that are audible to humans, due to the prohibitive costs of recording equipment and/or other practical issues (e.g., facility type, presence of visitors). Once potential calls of interest have been identified, the challenge is to highlight indicators of valence, not just arousal. Briefer (10) explains that this can be challenging because research on valence should compare calls emitted in both negative and positive contexts that are characterized by similar levels of arousal, and this can be difficult to find due to the fact that expressions of negative affect are typically more intense (see also 9).

It has become increasingly common for researchers to adopt a dimensional approach. As Briefer (10) notes, "this approach is useful for the study of animal emotions because it allows researchers to investigate differences between emotional states of low versus high arousal and of positive versus negative valence, without having to infer the specific emotion that the animal is experiencing" (p. 4). As described earlier, researchers can assess arousal level by integrating physiological biomarkers, such as measures of heart rate and respiration. Whitham and Miller (74) discuss technology and equipment that can be utilized to non-invasively assess autonomic nervous system (ANS) activity to provide insight into physiological functioning and arousal level. Ultimately, the goal is to highlight indicators of valence—whether they be particular call types or acoustic features.

Previous studies on both human and non-human mammals have provided evidence of vocal correlates of valence. When summarizing the literature for non-human mammals, there is growing evidence that: (1) a shift in valence is associated with a change in call type, (2) vocalizations emitted in positive contexts tend to be shorter in duration, and (3) fundamental frequencies may shift lower or higher when a particular call type is emitted in both positive and negative situations (10). This means that, for a given study species, researchers should aim to determine whether: (1) specific call types are more likely to occur (or exclusively occur) in certain contexts, (2) call duration varies when comparing vocalizations produced in negative versus positive contexts, and (3) calls of interest that are produced in both negative and positive contexts vary in terms of acoustic structure. Therefore, as a first step, a concerted effort should be made to analyze calls produced during situations/events known to be positive or pleasurable, as well those known to be negative or aversive. For example, are certain vocalizations more likely to be produced while feeding, playing, or receiving grooming? Are other vocalizations more likely to occur while initiating/receiving threats or aggression? Does call production vary by age/sex? Even if the researcher does not have the ability to analyze the duration or acoustic structure of calls, it should be possible to determine whether call types and rates vary across contexts. If the resources are available, researchers also can examine whether other parameters might be indicative of valance for the species of interest. Indeed, research on humans has demonstrated that vocalizations associated with positive affect tend to be characterized by certain features including, narrower frequency ranges, lower amplitudes, higher formants, less spectral noise, and an earlier position of maximum peak frequency (43, 45, 46, 132; reviewed by 10).

Overall, there is great potential for using vocal behavior to assess and monitor the emotions of individual animals. Vocalizations are well-suited for investigating the expression of an animal's inner state. As opposed to facial expressions or most behavioral states, vocalizations can communicate information to numerous individuals simultaneously, even if they are not in close proximity. Indeed, sound generally travels around features in the environment and carries long distances, though the features of certain settings (e.g., zoo enclosures with glass panes or lush vegetation) may present challenges when studying acoustic activity (8, 10, 11, 13, 128). Fortunately, such challenges can be overcome by introducing multiple microphones into the enclosure and arranging them strategically. Another benefit to studying vocal behavior is that call types and acoustic structure can change quickly to accurately reflect the caller's current state. While this means that many vocalizations can serve as honest, reliable indicators of short-term emotions, vocal activity may not be as helpful for gaining insight into an individual's long-term affective states or mood. Vocal behavior can still be a valuable indicator. For instance, if baseline data are available, vocalizations can be: (1) monitored to evaluate responses to changes in the environment and/or routine, or (2) tracked regularly to proactively highlight potential shifts in welfare status.

Indeed, vocal activity is an ideal measure to be integrated into welfare monitoring schemes for animals living under professional care, as vocal indicators can be tracked continuously and non-invasively. For some species, vocalizations may even be a better welfare indicator than some traditional measures, as changes in vocal activity (e.g., rates of production) may last longer than changes in appetite or other behaviors (64). Jones et al. (131) note that acoustic activity is underutilized in welfare research—particularly for aquatic animals—and promote the use of acoustic monitoring systems (e.g., WAMS). Real-time systems can trigger an alert, after which changes in call rates (for the group or individuals) can be compared to data from veterinary exams and behavioral observations. The authors note that these sorts of systems have the potential to: (1) detect instances

of aggression, early signs of illness, and anthropogenic/environmental sound disturbances and (2) determine whether a certain level of "communicative chatter" may serve as, "a positive signal of the 'status quo'" (p. 231). Given recent technological advances, call identification algorithms can even be applied to detect certain types of vocalizations (e.g., distress calls) or the real-time discrimination of valence (29, 126). Of course, even if researchers are unable to utilize recording equipment or bioacoustics software, ongoing behavioral monitoring can be conducted. Ultimately, animal care professionals can examine vocal behavior (even simply call rates) to highlight potential welfare issues and to proactively intervene by introducing changes to the environment and/or routine.

Finally, one of the most appealing reasons for integrating acoustic vocal behavior into studies of affect is that vocalizations allow for an emphasis on positive welfare. Indeed, while welfare studies traditionally have focused on negative indicators, the presence of positive affective states may be more relevant to welfare assessments than the absence of negative affective states (3, 9). Fortunately, many species' vocal repertories include calls that are associated with positive affect—from laughing gorillas to chirping rats to purring cheetahs (10, 79). We also recommend that welfare researchers investigate the vocalizations emitted by individuals facing challenges (e.g., novel enrichment such as puzzle feeders) designed to provide stimulation and promote natural behaviors (e.g., exploration, object manipulation). The vocalizations emitted by individuals in these situations—which are assumed to be stimulating and beneficial—may allow us to gain insight into how animals respond to eustress and short-term stressors.

Ultimately, the goal is to identify reliable, valid indicators of emotional valence for the species of interest—whether they be particular call types, vocalization rates, and/or acoustic features—and to integrate these with behavioral and physiological welfare measures. Vocal activity can then be monitored for individual animals continuously across various contexts and to evaluate responses to both acute and chronic stressors. A comprehensive welfare monitoring toolkit allows for researchers to not only conduct baseline monitoring but to also evaluate the impact of animal introductions, transfers, and changes to the husbandry routine or environment.

6 Conclusion

While the field of animal welfare science continues to expand, there is a need to identify indicators of affective state, and especially emotional valance, for most species. The human literature, as well as studies on various non-human mammals, have demonstrated that vocalizations can serve as valid indicators of short-term affect. Researchers working in zoological facilities, agricultural settings, companion animal shelters, and wildlife sanctuaries could greatly benefit by integrating measures of acoustic activity (e.g., vocalization rate, duration, acoustic structure) into systematic welfare studies, as well as ongoing monitoring schemes for individual animals. Having the ability to detect changes in vocal indicators of valence would allow welfare scientists to intervene when an individual's welfare seems compromised and to make informed management decisions. Incorporating measures of positive affect is vital, as the lack of negative behaviors alone does not imply that an individual is experiencing pleasurable states [e.g., (9)].

Ultimately, combining vocal indicators of affect with traditional welfare measures can help researchers conduct more comprehensive assessments of individual animal welfare, and in particular, will allow for a focus on positive welfare. Other indicators that can be incorporated in this toolkit include physiological biomarkers of welfare (e.g., measures of cardiac activity, cortisol/corticosterone) and various behavioral states and events. In the future, acoustic activity could be monitored continuously to detect shifts in welfare status or to assess the effects of animal transfers, introductions, and changes to the husbandry routine or environment.

Author contributions

JW: Conceptualization, Writing – original draft, Writing – review & editing. LM: Conceptualization, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

The authors are grateful to Dr. Sathya Chinnadurai and Tim Snyder for their ongoing support.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Veterinary Science

TYPE Review
PUBLISHED 12 March 2024
DOI 10.3389/fvets.2024.1370909

OPEN ACCESS

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RECEIVED 15 January 2024
ACCEPTED 23 February 2024
PUBLISHED 12 March 2024

CITATION
Ghimire R, Brown JL, Thitaram C and Bansiddhi P (2024) Comparison of animal welfare assessment tools and methodologies: need for an effective approach for captive elephants in Asia.
Front. Vet. Sci. 11:1370909.
doi: 10.3389/fvets.2024.1370909

Comparison of animal welfare assessment tools and methodologies: need for an effective approach for captive elephants in Asia

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Welfare is a fundamental aspect of animal management and conservation. In light of growing public awareness and welfare concerns about captive elephants, there is an urgent need for comprehensive, globally coordinated efforts for Asian elephants *(Elephas maximus)* that participate in religious, logging, or tourist activities in range countries where the majority reside, and where welfare issues have been identified but not addressed. This review provides a comparative analysis of available animal assessment tools. Each offers distinct features for assessment that allow institutions to select criteria for specific needs and available resources. Most are applied to general animal welfare assessments, although some are tailored to particular species, including elephants. The tools span diverse formats, from digital to primarily paper-based assessments. Assessments operate at individual and institutional levels and across multiple welfare domains. Methodologies rely on keeper ratings or expert evaluations, incorporate numerical scoring and Likert scales for welfare grading, and encompass inputs including behaviors, health, and physiological indicators. For tourist camp elephants, one challenge is that the tools were developed in zoos, which may or may not have application to non-zoological settings. Digital tools and assessment methodologies such as keeper ratings face logistical challenges when applied across tourist venues. As with any tool, reliability, validity, and repeatability are essential and must address the unique welfare challenges of diverse captive settings. We propose that a holistic, context-specific, evidence-based, and practical tool be developed to ensure high elephant welfare standards in non-zoological facilities throughout Asia.

KEYWORDS

animal welfare, captive elephant, welfare assessment, framework, tourist camp, zoo

1 Introduction

Animal welfare is a multifaceted concept that increasingly focuses on the cumulative physical, psychological, and behavioral states of individual animals (1). It encompasses scientific, ethical, economic, cultural, and religious dimensions with varying perspectives among scholars (2, 3). Initially, animal welfare science focused on enhancing the welfare of production and laboratory animals (4, 5). The Farm Animal Welfare Advisory Committee (FAWAC) took a significant step in 1965, developing the Five Freedoms model to address farm animal welfare concerns (6). It stated that animals should be free from hunger, discomfort, pain, and fear, and able to express natural behaviors. The model dominated discussions on animal welfare in Europe for decades (7), serving as a comprehensive framework while acknowledging the operational constraints of the livestock industry (8). However, criticisms surfaced, questioning its practicality and minimal emphasis on positive welfare experiences (9), prompting the development of alternative frameworks. The Five Domains Model offers a holistic approach focusing on affective terms and recognizing the subjectivity in measuring mental experiences (10). This emphasis on mental well-being aligns with broader ethical, policy, and legal considerations in contemporary animal welfare science. The model integrates the concept of agency within Domain 4 (Behavioral Interactions), enabling the evaluation of animal engagement in voluntary, self-generated, and goal-directed behavior (11) and human-animal interactions (10). Widely accepted in farm and zoo communities, the Five Domains Model has been adopted by organizations like WAZA (12) and the Zoo and Aquarium Association, Australasia (13) to uphold high welfare standards. However, to effectively utilize the model as a welfare assessment tool, attention should be given to using well-validated measures, ensuring transparency in expert panel selection, and implementing a clear welfare grading system (14).

Numerous welfare assessment frameworks have emerged by incorporating the Five Freedoms and Five Domains models. For example, Welfare Quality builds upon the Five Freedoms Model and integrates scientific expertise and ethical considerations from various stakeholders, including the general public, industry, and political bodies, to evaluate welfare (4). It prioritizes animal-based measures and follows a bottom-up approach, assigning scores based on four crucial principles: proper nourishment, suitable housing, good health, and appropriate behavior. These principles serve as the basis for evaluating overall welfare, and their scores are combined to determine the final assessment. The Opportunity to Thrive Program flips the concept of the Five Freedoms to focus on achieving a positive welfare state, with a particular emphasis on reintegrating animals back into their natural habitats (15). The framework offers a comprehensive method for managing animals, incorporating formulated diets, environmental design, healthcare, enrichments, choice and control, and access to species-typical behavior. These inputs ultimately aim to achieve desired outputs, resulting in an overall animal welfare assessment. A 24/7 approach was proposed to evaluate zoo animal welfare, utilizing the 12 welfare assessment criteria from the Welfare Quality framework (16). This approach considers the natural history, biology, ecology, diet, habitat, social structure, and activity patterns of animals throughout both day and night, providing a thorough understanding of their welfare. Finally, the Universal Animal Welfare Framework is an institutional-level welfare assessment framework based on the Five Domains Model (17). Developed by the Detroit Zoological Society in 2015, it examines zoo practices, policies, resources, and measures related to housing, routine, and behavior.

Both species-specific and species-general welfare assessment tools have utilized these welfare models and frameworks. Generalized tools work under the assumption that animals have the same basic needs, so management should be based on natural history. However, these tools face challenges in addressing species-specific nuances. A few have been developed for specific species and include the giant pacific octopus (*Enteroctopus dofleini*) (18), pygmy blue-tongued skink (*Tiliqua adelaidensis*) (19), bottle-nosed dolphin (*Tursiops truncates*) (20), waterfowl (Anseriformes) (21), dorcas gazelle (*Gazella dorcas*) (22), and elephant (*Elephas maximus*) (23).

These offer a refined and more precise evaluation of animal welfare by tailoring assessments to the unique needs, behaviors, and physiological aspects of a particular species (24). For some species with special spatial, environmental, social, or cognitive needs, a "one-size-fits-all" strategy to assess welfare may not be appropriate; rather, species-specific (25, 26) and if possible, context-specific assessment tools are needed.

Elephants, characterized by their large body size, complex social lives, varied food requirements, and extensive wild home ranges (27–29), pose challenges to meeting physical, psychological, and physiological needs in human-created environments (30). Ensuring good welfare for these animals involves allowing some degree of choice and control. Additionally, wild elephants spend about 80% of the time foraging and are highly social (31). Denying these freedoms can result in maladaptation, chronic stress, poor welfare (32, 33), and abnormal stereotypic behaviors (34, 35). Good zoos provide health care, safety from predation, and food security (30) and aim to meet exercise (36), foraging, and social complexity (37) needs. However, high mortality, low birth rates, limited reproduction, and health problems continue to hamper zoo elephant population sustainability (38–42), igniting worldwide concerns over animal welfare (43, 44). In 2016, a series of epidemiological studies of elephants in North American zoos revealed problems associated with ovarian acyclicity (45), health and musculoskeletal function (46), stereotypic behaviors (35), and high body condition scores (47). A similar set of studies on tourist elephants in Thailand found similar problems associated with elevated stress hormones (48), excessive body condition and metabolic derangements (49), and stereotypies (50). Finally, surveys of thousands of elephants in hundreds of tourist venues across Thailand, India, Nepal, Sri Lanka, Laos, Cambodia, and Malaysia suggest that more than half (63%) are kept in inadequate conditions (51–53). There is little doubt that comparable situations exist for logging (54), temple (55, 56), and circus (57) elephants as well. Thus, while the problematic state of captive elephant welfare across diverse conditions is now well-known, the solutions have proven far more elusive.

Considerable attention has been directed toward improving zoo elephant welfare, while the unique conditions and challenges faced by captive elephants in non-zoo settings are often overlooked (58). There are over 14,000 captive Asian elephants outside traditional zoo environments across 13 range countries, primarily in tourist or logging camps and temples (58). There are notable differences in the management of zoo and camp elephants. Zoo elephants are typically managed in protected contact systems, minimizing direct interaction with humans and other practices that adhere to standardized regulations (59). Staff are responsible for feeding, bathing, training, and veterinary care; however, because of limited space, socialization

and exploration can be limited. In contrast, camp elephants are often managed in free contact where elephants and people share the same space, including with tourists (60). Daily routines involve tourist-related tasks such as shows, riding, walking, bathing, feeding, or observation (60). Some elephants participate in cultural activities like religious rituals and festivals (61). Welfare can be better in camps situated in natural environments, with forests and rivers providing more natural foraging and exercise opportunities than zoos (62). However, restraint methods like chaining and using an ankus (also known as a bullhook or guide) to control elephants are significant concerns (63). In addition, the lack of enforceable standards results in varied management practices across and even within camps (64), which ultimately poses challenges in addressing the welfare needs of camp elephants. Animal activists continually voice concerns regarding the welfare and management of tourist camp elephants (65, 66). Thus, there is a need for a holistic, evidence-based welfare assessment approach to identify potential welfare risks, inform management decisions, and record welfare changes over time (67, 68). It also can contribute to elephant welfare standardization and policy-making processes crucial for properly managing elephants in range countries.

This review examines available generic and elephant-specific welfare assessment tools and methodologies and discusses applicability to tourist camp elephants (Tables 1, 2). While there have been several reviews of animal welfare frameworks (24–26), to our knowledge, this is the first overview of welfare assessment tools specific to elephants. Predefined criteria guided the selection of welfare assessment tools for this review to ensure a representative and comprehensive overview. Those included relevance to captive elephant welfare, recognition and adoption in the scientific community, and diversity of approaches. The featured tools were carefully chosen to provide readers with meaningful insights into the diversity and applicability of current welfare assessment practices for captive elephants, acknowledging that the selection may not encompass every existing tool. Our ultimate goal is to synthesize a new welfare assessment tool specific to elephants used in tourism, considering the strengths and limitations of existing tools and challenges faced by tourist camps.

1.1 Species general welfare assessment tools

1.1.1 ZooMonitor

ZooMonitor was developed by the Lincoln Park Zoo as a simple, software-based online tool to record the behavior and space utilization of individual animals using a digital device (72, 73). The tool is designed to examine activity budgets and behavior diversity across multiple zoo species. It allows the user to upload a map of animal habitats and evaluate space use over time. It facilitates 24-h systematic behavioral and social interaction monitoring and is flexible enough to be used with in-person observations or CCTV footage. The tool is continuously updated across iOS, Android, and Windows platforms. ZooMonitor has been adopted by over 200 institutions (72) and used in pygmy hippos (*Choeropsis liberiensis*) (73), penguins (Spheniscidae) (74, 75), chimpanzees (*Pan troglodytes*) (76), Madagascar giant hognose snakes (*Leioheterodon madagascariensis*) (77), tigers (*Panthera tigris*) (78), elephants (79), Japanese macaques (*Macaca fuscata*) (80), and others.

1.1.2 WelfareTrak

WelfareTrak, designed by the Chicago Zoological Society, is a user-friendly animal-based monitoring tool that relies on weekly keeper assessments of individual welfare (81). The tool is based on the concept that animal keepers are the most familiar with individual animals and can detect subtle behavioral changes. The welfare assessment sheet consists of 10 animal-based measures, including physical health (e.g., body condition), positive (e.g., calm-relaxed), and negative (e.g., self-mutilating) behaviors that are scored on a 5-point Likert scale. The quantitative scoring and flagging system of WelfareTrak allows organizations to set standards for animal care, track alterations over time, and objectively assess the efficiency of management practices and the effects of varied settings. The tool has been used successfully in many species including, but not limited to black rhinos (*Diceros bicornis*) (82), cheetahs (*Acinonyx jubatus*) (83), bears (Ursidae) (84), and western lowland gorillas (*Gorilla gorilla gorilla*) (85).

1.1.3 Zoological Information Management System (ZIMS) for Care and Welfare

ZIMS, managed by Species360 (Minneapolis, MN, United States), is a global database that manages data records for zoo and aquarium members. It is utilized by over 1,300 captive institutions in 102 countries for animal management and conservation (86). In addition to clinical and studbook databases, ZIMS has a module to record data related to animal welfare. The Care and Welfare module within ZIMS utilizes a welfare assessment strategy implemented by WAZA (12) based on the Five Domains Model. With elephants, ZIMS has been used to evaluate female social contexts (87), survivorship (88), and hormone cycle patterns (37). At the taxonomic level, each institution can specify parameters and assign anticipated values or ranges to each indicator within a domain. It offers data storage, record-keeping, and global sharing of life history, species biology, and management records. International recording and sharing of information make multi-institutional studies possible, eliminating the constraints of limited sample size in captive settings.

1.1.4 Welfare Discussion Tool (WDT)

The Lincoln Park Zoo developed the WDT for regular assessments of their collection of animals (89). It includes 41 items containing input (resource-based) and output (animal-based) measures related to behavior, endocrine activity (using non-invasive samples such as feces, swabbing skin in amphibians, etc.), husbandry and management practices, keeper interactions and observations, physical appearance, visitor interactions, and training programs. The measures are quantitatively scored on a 4-point scale (2 strongly disagree; 1 moderately disagree; +1 moderately agree; +2 strongly agree); all items also have an option of IDK (I do not know) and NA (not applicable). In two open-ended questions, raters are asked to recommend three improvements for animal welfare. The WDT assessment is conducted on each individual once per calendar year by three raters: (1) curator or manager, (2) animal caretaker, and (3) animal expert. The raters complete the assessments over 2 weeks and meet for discussion, after which the ratings are entered into the Lincoln Park Zoo's animal records software. While ZooMonitor has provided systematic behavior observation to gain data-driven insights from built-in graphs and reports, WDT presents a comprehensive assessment approach, inter-rater reliability across three raters, quantitative scoring, and regular

TABLE 1 Summary of available welfare assessment tools.

Tool	Developer	Online or paper-based	Assessment level	Measures used	Assessment methodologies
ZooMonitor	Lincoln Park Zoo	Online	Individual	Behavioral activity budget and diversity, space use	Observations using camera traps, CCTV footage, or in-person observations
WelfareTrak	Chicago Zoological Society (CZS)	Paper-based	Individual	Ten animal-based measures including physical health and behavioral indicators	Keeper-based ratings using 5-point Likert scale
Zoological Information Management System (ZIMS) for Care and Welfare	Species360	Online	Individual and Institutional	Based on the Five Domains model: • Nutrition • Environment • Health • Behavior • Mental health	Information gathering and sharing application Users select indicators and grading scales for welfare assessments based on species requirements
Welfare Discussion Tool (WDT)	Lincoln Park Zoo	Online	Individual and Institutional	41 resource and animal-based welfare measures	4-point scale (2 strongly disagree; 1 moderately disagree; +1 moderately agree; +2 strongly agree) Assessments conducted by: • Curator or manager • Caretakers • Animal experts
Animal Welfare Assessment Grid (AWAG)	Wolfensohn et al. (69)	Online	Individual	Modified Five Domains model: • Physical • Psychological • Environmental • Medical procedures	Keeper-based rating using a 10-point numerical scale
Animal Welfare Risk Assessment Process (AWRAP)	Sherwen et al. (67)	Paper-based	Institutional	Modified Five Domains model: • Environment (physical/social) • Behavior • Physical health/nutrition • Husbandry	Keeper-based rating using a scale of 0 (highest overall welfare risk) to 2 (lowest overall welfare risk)
Ackonc-Animal Welfare Assessment (AWA)	Racciatti et al. (70)	Paper-based	Individual and Institutional	Modified Five Domains model: • Nutrition • Environment • Health • Behavior / mental state	Keeper-based rating using a 3-point scale (A - normal/no observable welfare risk; B - mild deviation/welfare risk; C - Severe deviation/welfare risk)
Wild Welfare Animal Welfare Collection Assessment	Wild Welfare	Paper-based	Individual and Institutional	Based on the Five Domains model: • Nutrition • Environment • Health • Behavior • Mental health	Expert-based measures are scored as • Unacceptable • Questionable • Acceptable
Elephant Behavioral Welfare Assessment Tool (EBWAT)	Elephant Welfare Project under the British and Irish Association of Zoos and Aquariums (BIAZA)	Online and Paper-based	Individual	Qualitative Behavioural Assessment (QBA) and Behavioral Ethogram containing daytime and nighttime activity	Keeper-based rating using a Likert scale with responses ranging from 'never' to 'more than once per day' where appropriate and utilized various numbers of response options based on the expected frequency of that behavior

(Continued)

TABLE 1 (Continued)

Tool	Developer	Online or paper-based	Assessment level	Measures used	Assessment methodologies
Elephant Welfare Initiative (EWI)	Association of Zoo and Aquariums (AZA) Elephant Taxon Advisory Group	Online	Individual and Institutional	Based on the findings of multi-institutional epidemiological studies conducted in North America Resource-based measures (inputs) include housing features and management practices; animal-based measures (outputs) include behavior and physical health	Resource-based measures presented as logos indicating how goals were met during the day (sun logo), during the night (moon logo), or both Values indicate the percentage of each behavior observed Body condition score based on Morfeld et al. (47) Data based on direct observation by EWI members (experts)
Captive Elephant Welfare Index	Gurusamy and Phillips (71)	Paper-based	Individual	Factors include enclosure substrate, group size, health care, enrichment, restraining the animal, enclosure type, exercise provision, enclosure size, interaction with keeper and training, enclosure environment, keeper knowledge and experiences, diet, keeper contact method, display duration, and enclosure security	Expert-based rating using different scales; e.g., group size (1–4), display duration (1–5), and exercise provision (1–6)
World Animal Protection (WAP) Assessment	Schmidt-Burbach et al. (51–53)	Paper-based	Institutional	Based on Five Freedoms and Welfare Quality Factors include mobility, hygiene and shelter, environmental noise quality, the naturalness of the environment, social interaction, diet, entertainment intensity, and animal management	Expert-based rating using a 5-point scale with 1 being severely inadequate
ABTA Animal Welfare Guidelines: Elephants in Captive Environments	Association of British Travel Agents (ABTA)	Paper-based	Individual and Institutional	Based on Five Freedoms and Welfare Quality 12 criteria under good feeding, good housing, good health, and appropriate behavior domain of Welfare Quality along with three additional criteria addressing animals in tourism	Factors are divided into bad or best practices
Guidelines on the Usage of Captive Elephants in Malaysia	Malaysian Association of Zoological Parks and Aquaria (MAZPA)	Paper-based	Individual and Institutional	Guidelines include better housing and care, no physical abuse, provision of positive reinforcement, and others	No specific scoring system Body condition is scored using a scale; 0–5 = emaciated, 6–10 = average and > 10 = fat or very good condition

discussion between raters on post–assessment period to positive management changes to improve animal welfare.

1.1.5 Animal Welfare Assessment Grid (AWAG)

AWAG was developed for assessing the welfare of primates in research institutions (69, 90) but has since been adapted for birds (91), western lowland gorillas (92), giraffes (*Giraffa camelopardalis*), scimitar-horned oryx (*Oryx dammah*), and large felids (tigers, leopards, and cheetahs) (26). Based on the Five Domains Model, the tool divides welfare measures into four categories: physical, psychological, environmental, and medical, and uses a 10-point scale for quantitative measures. This tool allows individual and group-level assessment and presents the welfare measures as numerical and visual (radial chart) data.

1.1.6 Animal Welfare Risk Assessment Process (AWRAP)

The AWRAP was built on the Universal Welfare Assessment Framework and uses five animal-based and 15 resource-based measures divided into the environment, behavior, physical health/nutrition, and husbandry (67). These measures are scored from 0 (highest overall welfare risk) to 2 (lowest risk) based on keeper assessments. An overall welfare score is calculated for each measure

TABLE 2 Strengths and limitations of available welfare assessment tools.

Tool	Strengths	Limitations or Challenges (focused on tourist camps)
ZooMonitor	• Continuously updated across platforms, including iOS, Android, and Windows devices • Flexible for in-person observation or CCTV footage • Allows 24/7 systematic behavioral and social interaction monitoring	• Relies on behavioral observations that may be too time-consuming for mahouts • Mahouts may have limited knowledge of elephant biology and behavior for proper assessment • Integration of husbandry records required for holistic welfare assessment requires expertise • Challenges in low-budget venues and non-English-speaking regions
WelfareTrak	• Quantitative scoring and flagging systems for setting standards and tracking alterations over time play a crucial role	• Integration of resource-based measures is necessary for holistic assessment and may be lacking • The subjective nature of mahout assessments may introduce bias
Zoological Information Management System (ZIMS) for Care and Welfare	• Holistic approach to welfare assessment using animal and resource-based measures • Facilitates global sharing of information and data storage • Allows users to specify parameters and select grading scales	• Challenges in low-budget venues and non-English-speaking regions • Constantly updating information in ZIMS is logistically challenging • Implementing ZIMS might reveal welfare issues and require costly improvements that conflict with a camp's profit-oriented approach, making them hesitant to adopt the system • Public disclosure of welfare records may lead to negative publicity affecting the reputation and business of tourist venues
Welfare Discussion Tool (WDT)	• Holistic approach to welfare assessment using animal and resource-based measures • Inter-rater reliability across three raters • Regular post-assessment discussion between raters promotes positive management changes	• Endocrinological assessment can be challenging • Assessment by three raters regularly is time and resource-intensive for low-budget tourist venues
Animal Welfare Assessment Grid (AWAG)	• Holistic approach to welfare assessment using animal and resource-based measures • Numerical and visual representation allows welfare changes over time	• Scores may not correspond with behavioral observation data, relying heavily on mahout assessments • Difficult to access software and requires expertise to present the data in the radar chart
Animal Welfare Risk Assessment Process (AWRAP)	• Includes benchmark scores for welfare comparisons • Holistic approach to welfare assessment using animal and resource-based measures	• Focuses only on institutional-level assessment • Predominantly focused on resource-based measures (75%) leading to welfare risk assessment rather than overall welfare assessment • Reliance on mahout ratings may introduce bias and subjectivity • Measures like safety from predators might not be relevant in the context of tourist camp elephants
Ackonc-Animal Welfare Assessment (AWA)	• Holistic approach to welfare assessment using animal and resource-based measures • Reliable and valid measures are used	• Reliance on mahout ratings may introduce bias and subjectivity • Limited evidence on widespread adoption and validation
Wild Welfare Animal Welfare Collection Assessment	• Holistic approach to welfare assessment using animal and resource-based measures • Includes "non-negotiables" and a pre-intervention audit survey to identify common welfare concerns	• Implementation might conflict with tourist venues engaging in practices against Wild Welfare's "non-negotiables."

(Continued)

TABLE 2 (Continued)

Tool	Strengths	Limitations or Challenges (focused on tourist camps)
Elephant Behavioral Welfare Assessment Tool (EBWAT)	• Use of reliable and valid measures • Specific to captive elephants	• No evidence of widespread adoption and validation of non-zoological institutions • Lacks resource-based measures essential for risk assessment across captive institutions • Not intended to compare the welfare of elephants across facilities • Feasibility, reliability, and validity tested in UK zoos and may not apply to larger sample sizes or different contexts • Relying on 24-h monitoring is impractical in tourist camps
Elephant Welfare Initiative (EWI)	• Holistic approach to welfare assessment using animal and resource-based measures • Provides real-time analysis at individual and institutional levels • Allows benchmarking and monitoring over time	• Labor and time-intensive input requirements • May require technical expertise for effective implementation
Captive Elephant Welfare Index	• Utilizes validated measures	• Focuses only on institutional-level assessment
World Animal Protection (WAP) Assessment	• Specific focus on tourist camps	• Assumption and subjective criteria may influence scoring • Lacks integral components such as reliable and valid measures, and recent advances in animal welfare • Focuses only on institutional-level assessment
Association of British Travel Agent (ABTA) Animal Welfare Guidelines	• Specific to non-zoological institutions such as tourist camps	• Lacks integral components such as reliable and valid measures, welfare grading system, and recent advances in animal welfare
Guidelines on the Usage of Captive Elephants in Malaysia	• Specific to non-zoological institutions including tourist camps in Malaysia	• Lacks integral components such as reliable and valid measures, welfare grading system, and recent advances in animal welfare

and compared to a threshold score, generated from the distribution of scores across 220 enclosures at three zoos, and a criterion for the lowest 5th percentile value is set. Enclosure values below that limit are designated "at highest risk" with immediate welfare action advised, leading to positive management changes and facility adjustments.

1.1.7 Ackonc-Animal Welfare Assessment (AWA)

Ackonc-AWA is a recently developed multi-species tool based on the Five Domains Model that integrates 23 animal-based measures, 19 resource-based measures, and three management-based measures that fall under five domains: nutrition, environment, health, and behavior/mental state (70). Keepers grade each measure on a 3-point scale (A-normal/no observable welfare risk; B-mild deviation/welfare risk; C-severe deviation/welfare risk). It was developed in Spanish and the name is derived from the native Andean word "ackoncahua", meaning sentinels. The tool has so far been tested on 14 individuals (10 mammals, two birds, and two reptiles) for reliability, validity, and feasibility.

1.1.8 Wild Welfare Animal Welfare Collection Assessment

Wild Welfare is a UK-registered charity focused on welfare training and assessments, creating global partners, and improving animal welfare legislation (93). They have developed a welfare assessment tool based on the Five Domains Model that is used to conduct facility audits composed of 110 questions related to environment, health, behavior, mental state, caretakers, record keeping, health and safety of staff, and financial responsibility. Each measure is scored by experts in captive management and welfare as (1) unacceptable, (2) questionable, or (3) acceptable to identify the most common welfare concerns. As of 2020, 11 zoos in seven developing nations (Brazil, Egypt, Libya, Indonesia, Thailand, Malaysia, and Vietnam) have completed animal care audits (94). Findings often indicate that animal behavior, positive mental states in animals, and human health and safety are all areas that require assistance. Wild Welfare lists several non-negotiables, stating that facilities must use only positive reinforcement techniques and not restrict animal movements, permit animal demonstrations detrimental to physical or psychological well-being, allow feeding by visitors, or permit unregulated breeding.

1.2 Elephant-specific welfare assessment tools

1.2.1 Elephant Behavioral Welfare Assessment Tool (EBWAT)

Among the few elephant-specific welfare assessment tools is EBWAT, which utilizes qualitative assessments of individual daytime

and nighttime behavior (23). It was developed as a paper-based tool but is currently available as an Android application. The assessment approach involves qualitative evaluations of elephant behavior based on: (1) rating demeanor on a scale of 1–12 in four sets of 1-min observation periods in a single day; (2) daytime observations of comfort, social interactions, resting, feeding and stereotypic behaviors during four sets of 5-min observations during the day over 3 consecutive days; and (3) reviewing of overnight video footage using 30-min scan sampling. The reliability and validity of the tool were tested on 63 elephants at five UK elephant-holding facilities and are now used by 11 UK and Irish zoological facilities (23).

1.2.2 Elephant Welfare Initiative (EWI)

The EWI is a software-based online tool endorsed by the Elephant Taxon Advisory Group of the AZA as a follow-up to a series of multi-institutional epidemiological studies conducted in North America (95). It uses resource-based measures (inputs), including housing features and management practices, and animal-based measures (outputs) of behavior and physical health. The tool uses a web-based software system that allows users to integrate demographics (age, sex, species), housing plans, 24-h daily monitoring, behavioral and body condition scoring tools, and produces a series of welfare reports. It provides real-time analyses at individual and institutional levels that assist in benchmarking and monitoring changes. However, labor and time-intensive input requirements and inconsistencies in data outputs have limited its use.

1.2.3 Captive Elephant Welfare Index

This tool is based on the concept that captive elephant welfare is related to multiple husbandry parameters (71). Ten elephant experts identified 15 welfare indicators: enclosure substrate, group size, health care, enrichment, restraint, enclosure type, exercise provision, enclosure size, keeper interaction and training, enclosure environment, keeper knowledge and experience, diet, keeper contact method, display duration, and enclosure security (96). Different numerical grading scales (1–6) are used to score each measure, which are combined to obtain a total score. These measures were validated by behavioral and physiological (urinary cortisol) measures in Asian elephants managed at three zoos and three sanctuaries. Elephants with low CEWI scores had higher urinary cortisol and exhibited more stereotypic behaviors.

1.2.4 Assessments by World Animal Protection (WAP)

Welfare assessments based on the Five Freedoms and Welfare Quality models have been conducted on thousands of elephants in tourist venues throughout southeast Asia (Thailand, Nepal, India, Sri Lanka, Cambodia, Laos, and Malaysia) by WAP (51–53). Through direct observations of facilities and interviews with staff, numerical scores are assigned to factors such as animal mobility, hygiene and shelter, environmental noise quality, naturalness of the environment, social interactions, diet, entertainment intensity, and animal management on a 5-point scale. Low scores are assigned if elephants are used for tourist activities like riding, bathing, or feeding, chains are used for restraint, and the mahout carries an ankus. However, those assumptions are subjective, raising questions about their validity without considering how the activities are conducted (97). Rating scores range between 1 and 10 and are calculated as follows: FS = (x/xmax)9 + 1, where FS = final rating score, x = husbandry score, and xmax = maximum achievable husbandry score.

1.2.5 ABTA Animal Welfare Guidelines: Elephants in Captive Environments

The Association of British Travel Agents (ABTA) is among the few accredited organizations that have developed guidelines for non-zoological captive elephant management and care (98). Through extensive multi-stakeholder consultations involving experts, scientists, zoological organizations, and NGOs worldwide, ABTA has formulated comprehensive guidelines to ensure the welfare of elephants engaged in tourism. These guidelines prioritize a holistic approach, aligning with the 12 criteria under the Welfare Quality and Five Freedom frameworks, encompassing feeding, housing, health, and behavior domains. The manual delineates negative (bad) and positive (best) practices, identifying key areas that significantly impact elephant welfare. Practices promoting proper diet, suitable housing conditions, adequate healthcare, minimal chaining, opportunities for social interactions, and controlled public feeding contribute to optimal welfare. Conversely, bad practices, such as inadequate diets, substandard housing, insufficient healthcare, excessive chaining, intensive tourist activities, and lack of social interaction opportunities, significantly compromise welfare. To reinforce these standards, ABTA urges trade bodies and organizations to consistently monitor and verify that elephant-holding institutions adhere to the prescribed requirements for management and care.

1.2.6 Guidelines on the Usage of Captive Elephants in Malaysia

The Malaysian Association of Zoological Parks and Aquaria (MAZPA) devised comprehensive guidelines specifically focused on captive elephants engaged in tourist activities across Malaysia (99). These guidelines cover a spectrum of practices, including performances, presentations, riding programs, and interactive sessions like feeding, photography, and bathing. MAZPA's directives strictly prohibit physical threats or punitive measures toward elephants during these activities and emphasize the importance of conditions that mitigate unnatural behaviors. To ensure elephant comfort, the guidelines stipulate a minimum chain length of 4 meters with durations of less than 2 h between performances and housing on soft natural substrates Regular access to food and water is mandated, highlighting the crucial aspect of sustaining elephant health and vitality. Elephant handlers need to be qualified and knowledgeable in elephant care and using tools like the ankus and chaining.

2 Discussion

Within the two main welfare models used today, Five Freedoms and Five Domains, a range of methodologies exist for comprehensive welfare evaluations. To satisfy accreditation criteria, zoos and aquariums regularly evaluate the welfare of animals under their care (89), often using tools designed for multiple species. Each tool offers distinctive features that often serve different functions, such as complete behavioral and space utilization monitoring of ZooMonitor, global data sharing features of ZIMS, numerical and visual data representation of AWAG, or reliable and valid captive elephant measures presented by EBWAT. Tools range from digital formats to more traditional pen and paper for data recording and monitoring. However, overall, the trend is for institutions to use digital tools and advanced technologies to improve welfare standards (100). The tools

differ in assessment levels, from assessments to understand individual variation to institutional level assessments that can inform on prioritization of resources and broadly benchmark progress in advancing welfare standards. These tools mostly rely on keeper ratings as a proxy for quantitative behavior assessments because keepers spend more time with the animals and can detect subtle changes that might be overlooked by others less familiar (81). Most also use a relative grading system; for example, AWRAP implements a 0–2 scale, Welfare Discussion Tool a 4-point scale, Ackonc-AWA a 3-point scale, and AWAG has a 10-point scale. Likert scales are also commonly utilized when evaluating behavioral indicators (25). ZIMS is flexible to allow users to select the grading in binary, numeric, and percentile values. Objective welfare scores allow the recording of welfare changes over time (25) and assist accreditation schemes in determining if an organization meets welfare requirements (67).

Across the tools, inputs range from observing behaviors to measures of health and stress indicators to provide comprehensive assessments across different welfare domains. Observational behavioral assessments emerged as a standard in all of the existing tools. Some tools use CCTV or cameras, while others rely on direct observations by keepers or other experts. In one study, ZooMonitor was used along with 18 closed–circuit cameras and five camera traps to record behavior states, habitat use, and social interactions of seven zoo Asian elephants (79). That study highlighted the benefits of combining ZooMonitor with other assessment methodologies for comprehensive welfare interpretations. Tools are increasingly using behavioral indicators associated with comfort, play, affiliation, foraging, and sociality to evaluate mental and overall welfare states, in addition to commonly used and validated negative welfare indicators like stereotypies, poor health and reproduction, and high mortality and morbidity (23, 44, 101, 102). To that end, the score sheet of WelfareTrak consists of positive (e.g., calm-relaxed) and negative (e.g., self-mutilating) behaviors. EBWAT includes stereotypies, social interactions, feeding, comfort, social behaviors, interactions with the environment, vocalizations, and others to measure mental health. AWAG also evaluates stereotypies, social affiliations, enrichment utilization, and responses to training as measures of psychological welfare.

Many tools also incorporate health evaluations as animal-based measures of physical condition. Stool and urine appearance, body coat condition, wounds, skin lesions, locomotion, micturition behaviors, general illness, teeth condition, and coat condition are all included in the health domain of Ackonc-AWA. Physical assessments in the AWAG include factors such as body condition scores, appetite, drinking and feeding behaviors, and activity levels, while the AWRAP tool includes body condition and an overall general health score, and the WDT overlays behavioral data with cortisol (feces, urine, etc.) analyses. In the case of elephants, cortisol or its metabolites can be measured in blood, saliva, urine, feces, and hair (103). Indeed, a study in India found zoos and sanctuaries with low welfare scores tended to have elephants with higher urinary cortisol and stereotypy rates (71). Immunoglobulin A (IgA) is among the novel biomarkers used as a positive welfare indicator and also in assessments of immune function (104). Like cortisol, IgA fluctuations can indicate positive and negative welfare states (105, 106) and be measured non-invasively. Combining analyses of glucocorticoids and IgA with behavioral indicators like stereotypies can further validate assessment findings (107, 108). Methods like allostatic load indexes are gaining attention because of their ability to capture cumulative stress (109), and so could potentially be used to predict mortality and morbidity risks. Other indices to consider could include evaluations of preference/avoidance, displacement, vocalization, startle/vigilance behaviors, salivary or urinary epinephrine, heart rate variability, and cardiovascular function.

Digital tools play a significant role in zoological institutions, enhancing efficacy, data visualization, and multi-institutional collaborations (81, 100). However, implementing these tools institutionally in non-zoological settings will be challenging. Elephant mahouts may have limited knowledge of technological devices to use ZooMonitor or WelfareTrak, and most camps do not have research staff or volunteers to input data. Thus, paper-based assessment methodologies might be more appropriate. It also can be challenging for low-budget venues in range countries to afford CCTV cameras and access to software to analyze data. In tourist camps, where elephants are engaged in activities like bathing, riding, and walking in natural forests (60), CCTV monitoring is impractical and could raise privacy concerns. Constantly updating information in digital tools like ZIMS could also be a logistical challenge for camp staff. Finally, most of these tools are only available in English, making them less useful for range countries.

The current reliance on keeper ratings or expert opinions in welfare assessment tools for captive elephants in range countries also has limitations. Although intimately familiar with their elephants, mahouts (i.e., elephant keepers) might not consistently identify stereotypic behaviors or have a comprehensive understanding of the full spectrum of elephant behaviors (50). Studies have highlighted instances where mahouts, despite their proximity to the animals, could not identify certain behaviors accurately, leading to discrepancies between direct observations and keeper assessments (92). Moreover, mahouts often face time constraints in non-zoological settings due to engaging in tourist interactions, impeding their ability to monitor behaviors continuously. The potential for positive bias in mahout ratings, influenced by personal attitudes and care for specific animals, also raises concerns about the objectivity of assessments (67). A more effective approach might involve a collaborative model that combines the expertise of mahouts and trained observers. This hybrid approach utilizes both perspectives synergistically, with mahouts offering unique insights into individual elephant social interactions and preferences. At the same time, trained observers conduct focused, objective behavioral assessments, especially when evaluating stereotypies.

Moving forward, there is a need to develop a new welfare assessment tool specific to elephants used in tourism. Tools should go beyond mere adaptability from zoo-centric models to incorporate components that address the specific dynamics, challenges, stressors, and ethical considerations found in tourist camps. The tool should integrate a balance of animal and resource-based measures and avoid the narrow focus on single behavior or health indicators (23, 55) to provide a comprehensive welfare risk assessment (67). With an increasing focus on using welfare assessment frameworks for developing assessment tools, the Five Domain Model can be adapted to develop the welfare assessment tool. Despite criticisms against the Five Domain Model (14), it is the most widely used model in animal welfare science and is important because of its focus on mental states. If limitations such as reliable and valid measures focusing on the overall mental and welfare state of captive elephants, and a structured welfare grading system are considered (14), the Five Domain Model can be adapted to develop a new welfare assessment tool. Previously established behavioral measures for captive elephants (23, 101, 102)

and welfare factors associated with tourist camps can be integrated and adapted for further testing. The tool must be rapid, adaptable, undemanding in resources, non-invasive, and easy to complete, considering financial limitations, feasibility, and ethical concerns associated with invasive techniques (23, 25). Impractical measures like cognitive bias that require experimental setups (26) and measures such as safety from predators can be omitted, acknowledging their minimal impact on captive elephants in tourist camps. Despite recent efforts to enhance efficiency through technology (100), the practical constraints of tourist camps necessitate a focus on direct observation and questionnaires with mahouts. In the case of developing countries, a lack of understanding and awareness of animal welfare among mahouts makes it more challenging (94). To address this, a tool should integrate the perspectives of both mahout and experts, ensuring a more comprehensive and objective evaluation of elephant welfare. The tool should be designed to be executed by a trained individual familiar with the methodology, metrics, and relevant evaluation tools, intending to expand training to allow stakeholders and medical staff for in-house evaluation and assessments. Ensuring the tool's validity, reliability, and practicality is paramount (23). Achieving validity involves integrating existing literature, expert consultancy, and adapting established and validated assessment measures (25). Reliability can be tested through inter-rater, reliability, repeatability, and internal consistency assessments. The tool should be able to track welfare changes over time, integrating objective and quantitative welfare scores. This integration facilitates the comparison of welfare levels for future evaluations, enabling institutions to meet accreditation. It provides a quantifiable means to interpret individual welfare states, reduce inter-observer variability, and the potential for intra- and inter-group comparisons to establish best practices in elephant welfare across diverse tourist camps. A range of factors, such as age, health status, reproductive status, and life history, need to be accounted for in welfare assessments of captive elephants. Animals of different ages may react differently to the same scenario or resource allocation (110). Having baseline data for specific age groups for later comparison will contribute to developing a credible tool (25). For example, in the U.S. most captive elephants have experienced at least one inter-zoo transfer (111), which is associated with stereotypic behaviors (35). Similarly, seasonality in cortisol or its metabolites is evident in African (112) and Asian (113) elephants and so must be considered when evaluating the physiological significance of fluctuations as stress indicators. For example, in Thailand tourist camp elephants, higher fecal glucocorticoid concentrations were observed during winter (November–February), presumably due to colder temperatures (49), but during an international travel ban in Thailand during the Covid-19 pandemic, the highest concentrations were in the rainy season, suggesting it is tourist activities that are the most likely cause of increased glucocorticoid excretion during the winter, high tourist season months (114).

Implementing tools developed by ZIMS (Care and Welfare module) and Wild Welfare might reveal issues that require costly improvements, conflicting with the profit-oriented approach of elephant tourism, making camps hesitant to adopt changes. Welfare concerns surrounding captive elephants in Asia encompass various activities such as the use of ankus, chaining, riding, performing in shows, logging work, training methods, weaning, participation in religious rituals or festivals, and even involvement in polo tournaments. These activities provoke international concern, but the upright dismissal of such practices could lead to tension between local communities and outside experts. Thus, establishing collaborations among all stakeholders is vital for informed management adaptations.

Addressing welfare challenges and implementing assessment methodologies also demands clear objectives, heightened awareness, robust legal frameworks, and collaborative endeavors involving governmental bodies (115). Organizations like the Asian Elephant Specialist Group (AsESG) (116), WAP, ABTA, and MAZPA are developing conservation action plans, guidelines, and manuals for elephants managed in range countries. However, governmental concerns are often overlooked. Thailand, for example, initiated efforts to improve elephant welfare in 2002 with welfare standards for elephant camps, later supplemented by additional standards in 2009 (64). However, compliance was low due to non-enforceability and limited incentives. Thailand passed the Cruelty Prevention and Welfare of Animals Act in 2014 to prevent cruelty and improve animal welfare, but it has yet to be implemented. The Asian Captive Elephant Standards (ACES) were created to promote the well-being of elephants in Southeast Asia but require sincere participation from elephant camps and strict welfare monitoring by governmental bodies (64). Hopefully, the elephant camp standards launched by the Thailand National Bureau of Agricultural Commodity and Food Standards implemented in August 2024 will bring positive changes regarding the welfare of elephants in tourist camps. Similar issues are evident in other regions, like India (56, 117, 118), where many captive elephants are kept in temples under dismal conditions. Unlike conventional zoo or sanctuary environments, these settings operate under distinct governance structures that are often less restrictive and more culturally influenced. Therefore, a tailored welfare assessment tool must navigate the delicate balance between traditional and modern welfare standards, recognizing the diversity of beliefs and practices surrounding captive elephant management.

3 Conclusion and future directions

Addressing the welfare concerns of elephants in non-zoological settings, particularly tourist camps, presents a pressing challenge. Existing animal welfare assessment tools, although flexible, often lack essential components for effectively monitoring and enhancing elephant welfare in these contexts. Many tools were initially designed for zoological settings, rendering them less practical for non-zoological environments. Digital tools and methodologies such as keeper ratings encounter difficulties when applied to tourist venues because mahout knowledge of elephant biology and behavior is more limited. Tools should consider critical factors like reliability, validity, practicality, and recent advances in animal welfare science for comprehensive assessments. By doing so, we can better identify welfare risks, inform management decisions, track welfare changes over time, and contribute to standardizing elephant welfare practices and policy-making processes in non-zoological settings. This review proposes that there is a need to develop holistic, context-specific, evidence-based, and practical assessment tools tailored to the unique needs of tourist camp elephants across Asia. Recognizing the limitations of current approaches, we are actively engaged in developing a novel assessment tool specifically designed for assessing the welfare of elephants in tourist camps. This initiative aims to fill the gaps identified in existing methodologies and promote higher welfare standards for elephants across Asian tourist venues. By employing a comprehensive and tailored approach, we aspire to foster positive welfare outcomes

for elephants and contribute to the broader efforts aimed at enhancing animal welfare across diverse captive settings in Asia.

Author contributions

RG: Conceptualization, Writing – original draft, Writing – review & editing. JB: Conceptualization, Writing – original draft, Writing – review & editing. CT: Conceptualization, Writing – original draft, Writing – review & editing. PB: Conceptualization, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This review was supported by Chiang Mai University, Chiang Mai, Thailand. This study was supported in part from the Smithsonian Conservation Biology Institute (SCBI, United States) through a grant from the Shared Earth Foundation and the Anela Kolohe Foundation.

Acknowledgments

RG is a graduate student in a Master's Degree Program in Veterinary Science at the Faculty of Veterinary Medicine, Chiang Mai University, under the CMU Presidential Scholarship.

Conflict of interest

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Veterinary Science

TYPE Review
PUBLISHED 09 May 2024
DOI 10.3389/fvets.2024.1398116

OPEN ACCESS

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RECEIVED 08 March 2024
ACCEPTED 12 April 2024
PUBLISHED 09 May 2024

CITATION
Sundman ER, Dewell GA, Dewell RD, Johnson AK, Thomson DU and Millman ST (2024) The welfare of ill and injured feedlot cattle: a review of the literature and implications for managing feedlot hospital and chronic pens. *Front. Vet. Sci.* 11:1398116. doi: 10.3389/fvets.2024.1398116

The welfare of ill and injured feedlot cattle: a review of the literature and implications for managing feedlot hospital and chronic pens

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By definition, ill and injured animals are on the negative valence of animal welfare. For beef cattle kept in feedlot settings, advances in cattle health management have resulted in a greater understanding and prevention of illness and injury. However, the management of cattle once they become ill and injured is an understudied area, and there are gaps in knowledge that could inform evidence-based decision-making and strengthen welfare for this population. The aim of this review is to provide a comprehensive overview of the acquired knowledge regarding ill and injured feedlot cattle welfare, focusing on existing knowledge gaps and implications for hospital and chronic pen management and welfare assurance. Ill and injured feedlot cattle consist of acutely impaired animals with short-term health conditions that resolve with treatment and chronically impaired animals with long-term health conditions that may be difficult to treat. A literature search identified 110 articles that mentioned welfare and ill and injured feedlot cattle, but the population of interest in most of these articles was healthy cattle, not ill and injured cattle. Articles about managing ill and injured cattle in specialized hospital ($n = 12$) or chronic ($n = 2$) pens were even more sparse. Results from this literature search will be used to outline the understanding of acutely and chronically ill and injured feedlot cattle, including common dispositions and welfare considerations, behavior during convalescence, and strategies for identifying and managing ill and injured cattle. Finally, by working through specific ailments common in commercial feedlot environments, we illustrate how the Five Domains Model can be used to explore feelings and experiences and subsequent welfare state of individual ill or injured feedlot cattle. Using this approach and our knowledge of current industry practices, we identify relevant animal-based outcomes and critical research questions to strengthen knowledge in this area. A better understanding of this overlooked topic will inform future research and the development of evidence-based guidelines to help producers care for this vulnerable population.

KEYWORDS

animal welfare, Five Domains, feedlot cattle, production animal medicine, sickness behavior, hospital pen, chronic pen, decision-making

1 Introduction

Animal health and welfare in food production systems is a priority for producers, retailers, and consumers (1–4). Maintaining animal welfare via promoting animal health involves many moving parts, including understanding illness and injury prevalence, minimizing illness and injury occurrence, properly treating illness and injury when it occurs, and supporting the animal during recovery. In the beef feedlot sector, ongoing research into these topics has and continues to make improvements in feedlot cattle health and welfare. Prevalence studies are essential for understanding how common a particular condition is and how it is distributed across populations of interest. Prevalence studies have been conducted on feedlot conditions such as digital dermatitis (5), lameness (6), *Mycoplasma Bovis* (an important respiratory pathogen for Bovine Respiratory Disease Complex [BRDC]); (7), and ruminal acidosis (8). These studies have important implications for planning health management and research needs. Vaccination is one of the most crucial tools for preventing feedlot cattle infectious disease (9). Effective vaccination programs to lessen disease occurrence include considerations for vaccine type, vaccination timing, and secondary risk factor management (10–14). Understanding risk factors associated with illness and injury is also vitally important. For example, BRDC is a complex disease with risk factors that include host immunity levels, environmental conditions, and bacterial and viral pathogens that can influence pathogen transmission and stress-induced susceptibility (15). Risk factors associated with feedlot cattle lameness include body weight, source, stocking density, percentage of forage in the diet, season, precipitation, and temperature (16). By knowing and understanding how these risk factors impact feedlot cattle illness and injury occurrence, producers can work to minimize their herd's exposure to these risk factors, thus decreasing their risk of illness or injury.

In contrast, managing individuals after illness or injury has occurred is a less studied topic that has considerable implications for cattle welfare. Feedlot audits such as those in the United States (U.S.) (17), Canada (18), and under development in Australia (19) include sections addressing ill and injured cattle populations housed in hospital or chronic pens, and the U.S. and Canada audits indicate that failure to euthanize critically ill/distressed or injured cattle in a timely manner is an egregious act of neglect that can result in audit failure. Additionally, the care of ill and injured cattle has important implications for beef production sustainability and future. The Global Roundtable for Sustainable Beef[1] has identified animal health and welfare among core principles necessary for a viable beef value chain. Ill and injured cattle management and decision-making has implications for the economic viability of feedlot operations, social license to farm, and sustainability of the feedlot and beef sectors over time. Thus, there is considerable synergy between enhancing ill and injured feedlot cattle welfare and supporting feedlot operation productivity and sustainability. However, despite the growing importance of ill and injured feedlot cattle management and welfare, evidence-based guidelines designed to strengthen the care and welfare of ill and injured cattle are lacking. Thus, the aim of this review is to provide a comprehensive overview of the acquired knowledge regarding ill and injured feedlot cattle welfare, with a focus on gaps of knowledge that exist and implications for hospital and chronic pen management and welfare assurance. To achieve this, we will outline the current understanding of the cattle that make up this population. Then, we will appraise the impacts of illness and injury on cattle welfare using the Five Domains Model (20) and suggest how this model can guide future research. Ultimately, a better understanding of this overlooked topic will inform evidence-based guidelines for best practices in managing ill and injured feedlot cattle to help producers support the welfare of this vulnerable population.

2 Literature review methodology

A literature review was completed to understand the current published scientific findings specific to welfare and management of ill and injured feedlot cattle. Three separate searches were conducted. The first search was designed to identify peer-reviewed papers reporting on the welfare of ill and feedlot cattle. The second search focused on identifying peer-reviewed papers reporting on managing cattle in feedlot chronic pens. The third search was then widened to include feedlot hospital pens and other specialty pens used to house ill and injured cattle. After the initial search, papers deemed to be irrelevant were removed using the following exclusion criteria: "dairy" in the topic (Web of Science) or Article title, Abstract, or Keywords (Scopus) of the paper, "other topic" (about feedlot cattle, but no illness or injury animal outcomes), or "other reasons" (species other than cattle, non-peer-reviewed sources, language other than English). Detailed methodology, such as the specific search terms and databases used, the number of papers excluded for each exclusion criteria, and the final results from these searches (total and by paper type) can be seen in Table 1.

In summary, 110 unique articles about the welfare of ill and injured feedlot cattle were identified. The inclusion criteria for this search were quite broad—any papers that mentioned welfare, feedlot cattle, and measured any illness or injury animal outcome or discussed applications for ill or injured cattle. Applying more specific criteria such as restricting the scope to studies explicitly conducted on ill or injured cattle would likely further decrease this number. The literature search also identified 12 unique articles about managing ill and injured cattle in specialized hospital-type pens and two about managing them in chronic pens. Due to the sparsity of papers identified in this literature search, this review was further supplemented by papers identified through other manual methods. These manual methods included searching through reference lists from the original results, performing less targeted literature searches, searching journals associated with feedlot production or medicine, and talking to North American feedlot cattle experts for paper recommendations. Due to the limited number of papers on ill and injured feedlot cattle, some published papers exploring these topics in dairy cattle that had been excluded from the literature results table are included within the discussion to provide a more holistic view of the state of the literature on ill and injured cattle management.

It is important to acknowledge potential sources of bias in our methodology. Limiting the literature search publications in the English language may have biased the results towards articles from English-speaking countries. Inclusion of the search terms associated with housing (i.e., feedlot and feedyard) likely excluded results from

1 https://grsbeef.org/

TABLE 1 Results from three literature searches on the welfare and management of ill and injured feedlot cattle.

	Raw Results (#)	Excluded for "dairy" topic[1] (#)	Excluded for "topic – other"[2] (#)	Excluded for other reasons[3] (#)	Total excluded (#)	# remaining by article type (including repeats)			# remaining by article type (excluding repeats)			Total relevant papers (#)
						Primary Research	Review	Other[4]	Primary Research	Review	Other[4]	
Search 1: ("cattle" OR "beef cattle" OR "calf" OR "calves") AND (feedlot OR "feed lot" OR feedyard OR "feed yard" OR "dairy") AND (welfare) AND (sick OR sickness OR ill OR illness OR injured OR impaired OR unhealthy OR invalid OR ailing OR diseased OR down OR downer OR downed OR wounded OR damaged OR disabled OR lame OR emaciated OR debilitated)												
Web of Science (All databases, topic search)	4,787	4,596	65	23	4,684	77	14	12	82	14	13	110
Scopus (Article title, abstract, keywords search)	553	529	8	0	537	13	3	1				
Search 2: ("cattle" OR "beef cattle" OR "calf" OR "calves") AND (feedlot OR "feed lot" OR feedyard OR "feed yard" OR "dairy") AND ("chronic pen" OR "chronic pens")												
Web of Science (All databases, topic search)	2	0	0	0	0	1	0	1	1	0	1	2
Scopus (Article title, abstract, keywords search)	2	1	0	0	1	1	0	0				
Search 3: ("cattle" OR "beef cattle" OR "calf" OR "calves") AND (feedlot OR "feed lot" OR feedyard OR "feed yard" OR "dairy") AND ("hospital pen*" OR "sick pen*" OR "specialty pen*" OR "railer pen*" OR "realizer pen*" OR "recovery pen*" OR "special needs pen*" OR "alternate pen*" OR "alternative pen*")												
Web of Science (All databases, topic search)	59	46	1	1	48	11	0	1	11	0	1	12
Scopus (Article title, abstract, keywords search)	35	25	0	0	25	10	0	0				

[1]Papers with "dairy" in the topic were removed using the "NOT" Web of Science advanced search option. Papers with "dairy" in the Article title, Abstract, or Keywords were removed using the "AND NOT" Scopus advanced search option.
[2]"Topic – other" was defined as papers about feedlot cattle that had no ill or injured animal outcomes.
[3]"Other reasons" was defined as papers about non-cattle species, with non-peer-reviewed sources, or in non-English languages.
[4]The "other" article type included conference proceedings, meeting abstracts, book chapters, or opinion/editorial material.

10.3389/fvets.2024.1398116

extensive housing systems and pasture-based systems. Additionally, while articles from outside of North America were identified during the literature search and included, the manual search methods may have been biased towards North American intensive feedlot systems.

3 Defining ill and injured feedlot cattle

3.1 Ill vs. injured cattle

According to the Merriam-Webster dictionary, the definition of impaired is "in an imperfect or weakened state or condition" (21). In this review, impaired cattle will be defined as those in weakened states or conditions compared to healthy, fully functioning cattle, regardless of the source (injury, disease, other) or severity (mild, severe, acute, chronic). The definition of "impaired" includes two main subcategories—"ill" (synonyms: sick, unwell) (22) and "injured" (synonyms: damaged, wounded) (23). Thus, ill cattle are considered as those not in good health due to disease or other pathological conditions and injured cattle as those with physical harm or damage to the body not attributed to disease.

According to the United States Department of Agriculture (USDA) National Animal Health Monitoring System's (NAHMS) Feedlot 2011 survey of the U.S. feedlot industry, the most common conditions in feedlot cattle in operations ≥1,000 head are: respiratory disease (16.2% of cattle), digestive problems (4.3% of cattle), acute interstitial pneumonia (2.8% of cattle), bullers (2.8% of cattle), lameness (1.8% of cattle), and central nervous system problems (1.1% of cattle; e.g. polio) (24).

3.2 Acute vs. chronic cattle

For this review, "acute" and "chronic" cattle are considered two separate subcategories of impaired cattle. Acute cattle are those with conditions that resolve within a short time (days or weeks), either successfully through recovery or unsuccessfully through mortality. Acute cattle are often treated in their home pen or may be temporarily housed in a treatment/hospital pen before being returned to their home pen. Conversely, chronic cattle are those with long-term conditions (weeks or months) that result from failure to recover in a timely manner. Chronic cattle are often treated multiple times and may be moved to a separate chronic pen after failed treatments.

Data regarding acute vs. chronic cattle prevalence is lacking in the published literature, and most data that is available pertains to respiratory disease. The USDA NAHMS 2011 U.S. feedlot survey data for feedlots ≥1,000 head (24) reported expected percentages of cattle for each final disposition (recovery, mortality, chronicity, and retreatment) after one, two, or three treatments for respiratory disease in two different weight classes (above or below 318kgs [700lbs] when placed). Regardless of weight class, approximately 16.2% of feedlot cattle were diagnosed with respiratory disease, and 87.5% of those were treated. Of those treated, greater than 80% of cattle recovered after one treatment (and hence were categorized as acute cattle according to our definition), and mortality rate after first treatment was less than 4%. Of the treated cattle, less than 15% received additional treatments, and additional treatments were often with a different product. Successful second treatment response was lower than the first treatment response (over 60%), and mortality for second treatment cattle was also higher (about 13%). Finally, a small percentage of cattle fail to respond to both the first treatment and second treatment. At this stage, producers may decide to pursue further treatment or other alternatives, such as railing (shipping for slaughter prior to reaching expected slaughter weight) after an appropriate antibiotic withdrawal period. The third treatment response rate (~40%) was lower than both the first and second treatment response rates, and the mortality rate (~30%) for third treatment cattle was also higher. This higher mortality rate can be expected and is perhaps due to factors such as infections with drug-resistant pathogens or because the disease has progressed to a severe point where the animal cannot adequately respond to the infection or recover their respiratory function (24). Additional treatments beyond the third treatment are not reported in the NAHMS data, but casual observation indicates that this population does exist at some feedlots.

Figure 1 presents these data specific to a hypothetical feedlot of 10,000-head of cattle <318kgs (700lbs) when placed, which have a higher respiratory disease morbidity (21.2%) and treatment (19%) rate than cattle ≥318kgs (700lbs) when placed (8.8% morbidity and 7.4% treatment rate). In summary, a 10,000 head feedlot with cattle placed at <318kgs (700lbs) will treat approximately 1900 cattle for respiratory disease. Of those 1900 cattle treated, 1744 (91.8%) will recover, 124 (6.5%) will die, and 69 (3.6%) will be considered chronic and railed within one, two, or three treatment events (these numbers are slightly above 100% of cattle, due to multiple responses in the NAHMS data). Relating these numbers to the original population of healthy cattle, 17.4% will be diagnosed with respiratory disease, treated, and recover. The expected mortality rate for respiratory disease would be 1.24%, with only 0.07% of cattle being diagnosed with respiratory disease, treated, and becoming chronic. The "total outs" (mortality + chronicity) from respiratory disease would be 1.93% of the original population. While this figure outlines cattle outcomes for respiratory diseases, some questions remain. For example, what happens to cattle that fall under each of these outcome categories, and how severely and how long is their welfare impacted? What are the implications for impaired cattle with conditions besides respiratory disease?

3.3 Dispositions and welfare considerations

There are four common dispositions that both acutely and chronically ill cattle may experience: recovery, railing, euthanasia, and unassisted death. For both the producer and the animal, the best-case outcome is recovery. Estimates for mortality rates in feedlots range from 1 to 2% (24–26), meaning that the vast majority of morbid cattle will recover (with recovery defined as not a mortality event). Precise numbers of ill or injured cattle that will fully recover are sparse. The NAHMS data indicates that of the 16.2% of cattle affected by respiratory disease, approximately 92–96% will recover after 1–3 treatment events (92% of cattle <318 kgs [700lbs] when placed and 96% of cattle ≥318 kgs [700lbs] when placed, respectively). The data also suggests that chronic cattle with BRDC (which often receive multiple treatments) may have decreased recovery rates. A descriptive epidemiologic report of chronic calves from a single Western Canadian feedlot in 1998 reported that 60% of calves in their chronic pen were returned to their home pen after an average recovery period of 30 days (27). More recently, a small study completed in Iowa

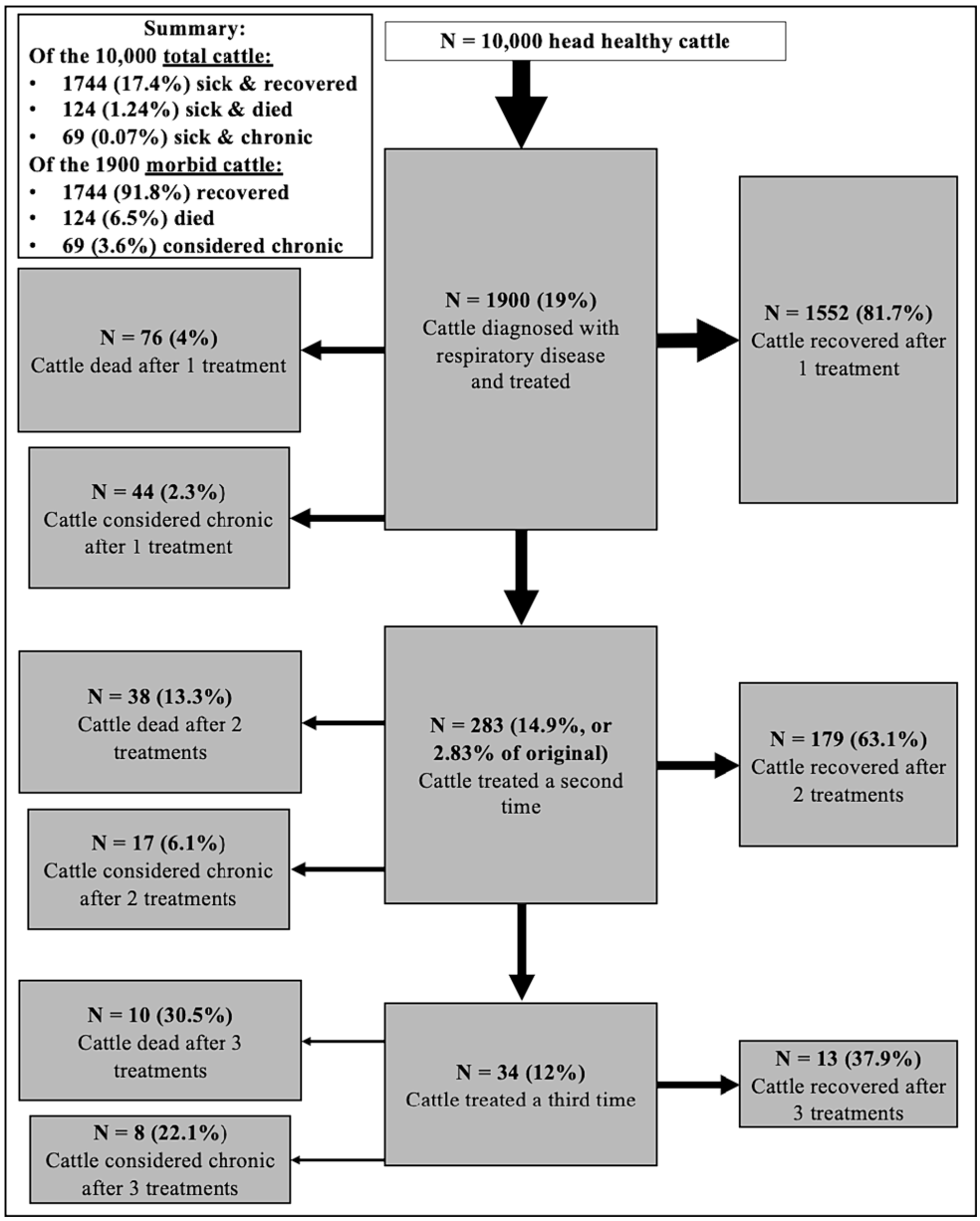

FIGURE 1
Dispositions for number (N) and percent (%)[1] of feedlot cattle diagnosed with respiratory disease after one, two, and three treatment events in a hypothetical feedlot of 10,000 head. All cattle were < 318kgs (700lbs) when placed. Adapted from USDA NAHMS[2] data (24) for US feedlots ≥1,000 head. [1]Percents may not add to 100 due to multiple or unspecified responses. [2]United States Department of Agriculture (USDA) National Animal Health Monitoring System (NAHMS) Feedlot 2011. [3]Considered chronic and realized (railed). Defined as cattle shipped for slaughter before reaching expected slaughter weight.

feedlots found that 83% of the calves that entered the chronic pen were either returned to their home pen or harvested from the chronic pen (28). While variable geographic and climactic conditions may explain some of these differences, the overall sparse and variable reports of cattle recovery rates in different feedlots suggests that animal recovery is also likely impacted by factors within the feedlots themselves, and more research is needed into what factors can impact animal recovery.

Railer cattle (sometimes called "realizer" cattle) are cattle sold before reaching their expected slaughter weight. Reasons for this failure to reach slaughter weight can include injury, chronic illness, poor performance, or a combination of these factors. The term "railer" stems from the ultimate endpoint– the rail at the packing plant. Cattle sent to slaughter as railers are expected to have a lighter-than-typical carcass weight, which leads to some losses on the initial investment (29). Very little data on the expected number of railer cattle in a feedlot setting is available. One source indicates that the expected proportion of cattle in a feedlot that will be railed is 0.42% (30). According to Terrell and colleagues, the leading diagnosis of railer animals is lameness and skeletal issues (47.83% of railers), followed by BRDC (43.48% of railers) and non-performance issues (8.7% of railers) (31). There is an economic incentive for producers to rail animals for partial value, but animal welfare considerations must also

be considered. Railer cattle that are ill and or/injured may be in a negative state of animal welfare due to their illness or injured state and may be experiencing negative mental states such as pain, distress, or feelings of malaise. The additional stressors involved with the railing process (such as transport, being sold at auction, and adapting to a new environment) may be exacerbated in ill and injured cattle compared to healthy cattle. Cattle should only be railer candidates if they are not in pain, can freely stand and walk, and are disease, drug, and chemical residue free (32). Cattle should also meet fitness for transport guidelines, such as those outlined by the American Association of Bovine Practitioners (AABP) and Canadian Food Inspection Agency fitness for transport guidelines (33, 34). Thus, when deciding whether to keep an ill or injured animal with the hope of recovery, market it as a railer animal, or potentially euthanize it if it is not fit for transport, there are welfare risks that need to be considered (35, 36).

Some animals may be so severely ill or injured that euthanasia is the best option. Guidelines for humane euthanasia of cattle are provided by AABP, Beef Quality Assurance, and the American Veterinary Medical Association in the U.S. and by the National Farm Animal Care Council in Canada. When deciding which animals are candidates for euthanasia, factors to consider include the animal's pain and distress, quality of life, likelihood of recovery, ability to get to feed and water, drug withdrawal time, economic considerations, condemnation potential, human safety, and diagnostic information (37–41). The AABP guidelines suggest that no more than 4 h should pass between the euthanasia decision and the euthanasia event (41). Euthanasia decisions are a key evaluation criterion in feedlot audits, including those created by the National Cattlemen's Beef Association in the U.S. and the National Cattle Feeders Association in Canada. Failure to euthanize a distressed animal in a timely manner is considered an egregious act of neglect, which results in an automatic audit failure (17, 18). This possibility of an audit failure from the identification of an animal in need of euthanasia that is not being properly handled highlights the importance of timely euthanasia and clear protocols to provide guidance about when and how to euthanize a severely ill or injured animal. While timely euthanasia is a crucial factor that can impact animal welfare, there is evidence that timely euthanasia may not always occur at necessary levels. Timely euthanasia is subjective, and data is limited; more guidance exists for dairy cattle than feedlot cattle. A 2020 review on timely euthanasia in the dairy industry concluded that timely euthanasia is a concern in the dairy industry and that more resources are needed to provide employees with the tools necessary to make these critical decisions (38). For example, dairy caretakers that underwent case-study based training for treatment decisions such as euthanasia stated that while they felt confident in making euthanasia decisions before the case study discussion, the training experience was still beneficial in improving their euthanasia decision-making skills (42). A 2019 survey of pen riders from 31 Texas feedlots indicated that feedlot pen riders are less confident in performing euthanasia than managers and veterinarians and were more likely to indicate that cattle are not always euthanized in a timely manner (43). Primary research focusing on clarifying ambiguity in euthanasia guidelines and increasing confidence in euthanasia decision-making by providing clear animal-based outcomes and defined endpoints are needed to ensure timely euthanasia of ill and injured feedlot cattle. Additionally, the practice of on-farm emergency slaughter (OFES) is used as an alternative to euthanasia in some countries for cattle that are unfit for transport but still fit for human consumption (44). While OFES is intended to prevent transportation of unfit animals while salvaging their meat (45), there is some controversy over whether OFES provides prompt relief (i.e., quickens or delays death) for injured animals (46, 47), and thus additional research in necessary to determine the welfare implications for cattle undergoing this process.

Unassisted death occurs when an animal dies without human intervention (i.e., in the absence of euthanasia). While unassisted deaths are more common than assisted deaths (euthanasia) in U.S. feedlots, overall mortality rates are low at only 1–2%. Hence, unassisted death is a relatively uncommon outcome overall for feedlot cattle. Unassisted death may result from acute conditions (e.g., heart failure, lightning strikes) or chronic conditions (i.e., chronic BRDC or lameness). A key welfare consideration for cattle that die unassisted is the severity and duration of suffering before a mortality event. This is especially true for chronically ill or injured cattle, since chronic impairments that have progressed to a state of severity where death is imminent are likely accompanied by severe welfare impairments (such as pain, distress, breathlessness, malaise, hunger, and discomfort). Minimal information on the prevalence of unassisted deaths in chronic cattle is available. One reason for this may be that some feedlots or sources may not indicate whether mortalities result from euthanasia vs. unassisted death. For example, Pollock and colleagues indicate that for chronic calves, 40% either died or were euthanized after a short recovery period of only 15 days (27), but does not further split this into unassisted deaths vs. euthanasia. The limited information available indicates that unassisted death is typically more common than euthanasia. A small study of 5 Iowa feedlots reported 14% mortality, and 3% were identified as euthanized. When asked, most feedlot managers responded that unassisted deaths were more frequent in their chronic pens than assisted deaths (euthanasia) (28). Research regarding factors that may lead to non-responsive cases, unassisted death, and animal-based outcomes that are indicators for immediate euthanasia is an area of need that could help clarify euthanasia guidelines, barriers to timely euthanasia and ultimately minimize the occurrence of unassisted deaths.

3.4 Behavior during convalescence

To understand why ill and injured cattle may benefit from specialized care and management, it is first necessary to outline how behavior differs between impaired and healthy animals. It is well established in the scientific literature and clinical practice that when an animal is ill or injured, its behavior will change. In the past, sickness behaviors were considered an undesirable disease effect. In a critical review in 1988, Hart described changes in animal behavior as a response to sickness not as a "maladaptive or undesirable effect of illness, but rather a highly organized behavioral strategy that is at times critical to the survival of an individual" (48). In other words, the function of sickness behavior is integrated with the innate immune response, which influences an animal's chances of recovery from illness. Research regarding sickness behavior as an adaptive response to disease in humans and other animal species has continued to grow [for more recent reviews, see (49–52)].

Activating the innate immune system is the first step of many that ultimately leads to a change in impaired animal behavior.

Neuroimmunoendocrine mechanisms behind sickness behavior have been an active area of study [e.g., (49, 50)]. To summarize, the innate immune system can be activated in response to infection with a pathogen, tissue damage, and other irritants (e.g., heat stress) (52). When an animal is infected with a pathogen, immune cells recognize molecular structures on the pathogen called pathogen-associated molecular patterns (PAMPs). When an animal experiences tissue damage, the broken cells produce alarmins. In both of these cases, sentinel immune cells such as dendritic cells, macrophages, and mast cells have receptors that can detect PAMPs or alarmins and will respond with the release of inflammatory cytokines, which are the primary agents that result in what we call sickness behaviors (50, 52). Four major cytokines are associated with sickness behavior: Tumor necrosis factor-α (TNF-α), Interleukin-1 (IL-1), Interleukin-6 (IL-6), and high mobility group box protein-1 (HMGB-1) (50, 53, 54). There is some evidence that other cytokines, such as interleukin-18 (IL-18) and interferon-γ (IFN-γ), may also play a role in sickness behaviors (50, 55). These inflammatory cytokines act on the brain to trigger responses that include physiological changes in the body (such as fever) and sickness behaviors.

Many behavioral changes occur that are considered sickness-related behaviors, and these behaviors are highly conserved across animal species. For a recent review on non-species-specific sickness behaviors, see (56). Most primary research on cattle sickness behavior has been studied in dairy cows with common dairy production diseases, such as hypocalcemia, ketosis, metritis, mastitis, and lameness. Sick dairy cows displayed increased resting/lying duration (57–59), decreased activity (60, 61), and decreased feeding behaviors [e.g., time at feeder, number of feeder visits, feed intake (59, 62–65)]. Sick dairy cows also expressed decreased duration ruminating (60, 61). Social behavior expression also decreased in response to sickness. For example, sick dairy cows performed fewer bunk displacements (64), fewer agonistic behaviors (65, 66), and less allogrooming (66). One study also reported that lame dairy cows were recipients of social licking by their pen-mates more frequently than non-lame dairy cows (67). Neonatal dairy heifer calves infected with Bovine Respiratory Disease Complex (BRDC) and neonatal calf diarrhea displayed decreased exploratory behavior when exposed to novel object and stationary human approach tests relative to healthy calves (68). When exposed to a low dose of bacterial endotoxin, dairy calves expressed sickness behaviors such as decreased rumination, decreased hay eating, decreased self-grooming, increased lying, and increased standing inactive (69). For a more extensive review of dairy cattle sickness behaviors, see (53). These sickness behaviors in dairy cattle may have some application for beef cattle. Nevertheless, research on sickness behaviors in beef feedlot cattle specifically, including male cattle, is necessary due to differences in genetics, nutrition, environment, and rearing that may impact these behaviors.

Primary research on feedlot cattle sickness behaviors is less extensive and primarily focused on BRDC. Cattle with BRDC display decreased activity (70–72), decreased feeding behaviors [e.g., lower dry matter intake, less time feeding, and less time near the bunk; (73–76), decreased rumination (70, 72), fewer lying bouts (71, 73), and increased lying duration (73)]. Cattle with BRDC may also groom less (73) and may have a lower pain threshold [hyperalgesia; (73)]. These general sickness behaviors may also be expressed in cattle with other common feedlot diseases, such as acidosis, pneumonia, digital dermatitis, and general lameness. For example, a review article on feedlot cattle with acidosis states that decreased feed intake is a consistent clinical sign of cattle with acidosis (77). Cattle with pneumonia spent more time lying down and less time eating than healthy counterparts (78). Cattle with digital dermatitis showed decreased rumination and increased inactivity (79). Like dairy cattle, there is evidence that diseased feedlot steers may receive more allogrooming than their non-diseased counterparts (80). There is also some evidence that water intake will change with disease and can be used to predict disease onset (81). Finally, level of parasitic infection (severity of disease) can impact the level of sickness behavior expressed by an animal (82–84).

3.5 Identifying and managing ill and injured feedlot cattle

Ill or injured feedlot cattle are identified by employees called pen riders. At a larger feedlot, pen riders are typically a separate group of employees responsible for checking pens and identifying ill or injured animals, sometimes from horseback (hence the term "rider"). At a smaller feedlot, while there may not be a designated "pen rider" job, there are still employees responsible for regularly checking cattle pens. Pen riding is a difficult task, and it requires excellent observation skills and knowledge of what to look for to identify individual ill or injured cattle in large groups of healthy cattle. Portillo provided a comprehensive description of the best practices in pen riding in U.S. feedlots, including how season, cattle excitability, and cattle risk status can impact pen riding strategies (85). Recent technological advances have also made identifying ill or injured cattle with technology feasible, although this is still a developing area (86). Once an animal is identified as ill or injured and in need of treatment, treatment strategies for that animal may vary depending on the disease identified, the etiology and severity of the disease, and the characteristics of the affected animal.

It is important to recognize that ill and injured animals are unavoidable in livestock production. While the ideal situation would be that all animals remain healthy, and producers, veterinarians, and researchers continue to strive for this goal, ill and injured cattle exist. Thus, when ill and injured cattle are identified, it is vital to manage them in a way that promotes positive and minimizes negative welfare while supporting their return to health. An ill or injured animal is inherently on the negative valence of animal welfare (experiencing a negative rather than positive state). Careful and thoughtful management practices can promote and support cattle welfare while they are impaired. The practices and types of pens used for housing and managing ill and injured cattle populations vary greatly between feedlots. For this review, any pen specifically designated to house impaired cattle of any kind will be defined as a pen in a "hospital pen system." Within a hospital pen system, there are three sub-categories of pen type: "hospital pen," "chronic pen," and "specialty pen." A hospital pen houses acute cattle for a short stay, and cattle have often been recently treated. The chronic pen typically houses chronic cattle for a longer stay compared to the hospital pen. Cattle in this pen often have been treated multiple times and may or may not receive additional treatments (87). Finally, a specialty pen is any pen that does not fit within the hospital or chronic pen designations. Examples of specialty pens in feedlots include the buller pen (which houses cattle

affected by buller-steer syndrome) and the realizer or railer pen (which houses cattle that will be shipped to slaughter before reaching market weight). Some facilities may have separate or combined chronic and railer pens, and others may have an additional extended-recovery pen or small pasture for animals that would typically be housed in a chronic/railer pen but may benefit from additional time in a recovery pen instead of being immediately railed once drug withdrawals are met. The number and types of pens in a feedlot hospital pen system will vary depending on the feedlot size and needs. Feedlot purchasing practices, such as a predominance of higher or lower-risk cattle, may also influence hospital pen systems. A large feedlot or one with a large high-risk cattle demographic may have enough morbid animals to support many pens in their hospital pen system for different types of impaired cattle. In contrast, a smaller feedlot or one that purchases lower-risk cattle may only have a single pen for all impaired cattle (87, 88).

4 The Five Domains and ill and injured cattle welfare

4.1 The Five Domains Model—an introduction

According to the Terrestrial Animal Health Code published by the World Organization for Animal Health (WOAH), animal welfare is defined as "the physical and mental state of an animal in relation to the conditions in which it lives and dies" (89). The Five Domains Model is a conceptual framework and tool for assessing animal welfare. Introduced by Mellor and Reid in 1994 (90), the Five Domains Model contains five areas (Domains): 1- Nutrition, 2- Physical Environment, 3- Health, 4- Behavioral Interaction, and 5- Mental State (Figure 2). The first four domains are considered physical/functional domains, as they focus on the internal physical state of the animal. Domain 5 is the mental state domain, as it considers the mental experience of the animal and how the aspects of the first four domains impact that animal's mental state. Therefore, the first four domains are filtered through the mental state domain to ask, "how do these functional domains impact the animal's subjective mental experience?" or more simply, "how do they make the animal feel?." The overall mental state of the animal as a cumulation of the impacts of the first four domains can then be used to assess the animal's current welfare state (20).

The first three domains (Nutrition, Physical Environment, and Health) are often referred to as the "survival-critical" domains, as they give rise to negative affect (Mental State Domain) critical to the animal's survival (such as breathlessness, thirst, hunger, pain, nausea, dizziness, and weakness) (91). Domain 4 (Behavioral Interaction) focuses on an animal's external physical and social environment and how behavioral interactions with the environment can impact welfare. These situation-based factors considered in Domain 4 reflect the cognitive responses of animals in different situations, such as being kept in impoverished environments, confronted by threatening situations, or otherwise restricted in their ability to engage in agency-related behaviors (20, 91). Agency is defined as an animal's ability to consciously engage in goal-directed behaviors, or more simply its ability to choose the behaviors it expresses (20). Situations where agency is impeded may cause negative affect (Mental State Domain) such as anxiety, fear, panic, frustration, anger, helplessness, loneliness, boredom, and depression. Situations where agency can be exercised may cause positive affect (Mental State Domain) such as calmness, engagement, excitation/playfulness, and confidence (91). There are three subcategories of the behavioral interaction domain: interactions with the environment, interactions with other animals, and interactions with humans (20).

4.2 Illness and injury within the Five Domains

Through the Five Domains Model lens, impaired welfare can stem from illness or injury associated with Domain 3 (Health) and subsequent impacts on Domain 5 (Mental State) through feelings of pain, malaise, weakness, breathlessness, nausea, and physical exhaustion. Furthermore, reduced feeding and drinking behaviors may occur from inappetence, reduced foraging motivation, or reluctance to compete at the feed bunk, which can lead to reduced feed and water intake (Nutrition Domain) and subsequent hunger and thirst (Mental State Domain). Similarly, ill and injured animals that develop a fever often display heat- or cold-seeking behaviors (Behavior Domain) which may be exacerbated by thermal extremes in the environment (Environment Domain), which can impact thermal comfort (Mental State Domain). Hence, the confluence of sickness behavior with the design and management of hospital and chronic pens has tremendous potential to impact cattle welfare and recovery positively or negatively.

In addition to the presence of illness or injury leading to impaired welfare, one must consider that the severity and length of the health impairment can also impact welfare (92). When grading the degree of welfare compromise in animals with "untoward organ-specific clinical signs with various effects," Mellor notes that animals with no clinical signs have no welfare compromise, animals with minor/short-lived clinical signs have "low" welfare compromise, animals with marked/short-lived or moderate/longer lived clinical signs have "marked to severe" welfare compromise, and animals with extreme clinical signs, followed by death while conscious have the most severe level of welfare compromise (92). This variation in welfare compromise from none to severe based on the length (short vs. long) and severity (minor vs. marked) of clinical signs of disease can be applied to acute vs. chronic feedlot cattle. Acute cattle tend to have a shorter duration of health impairment, but that short duration may be filled with more marked/severe clinical signs. In comparison, chronic cattle tend to have a longer duration of health impairment, where clinical signs may be less severe. Of course, these trends may vary on a case-by-case basis. For example, an individual may initially experience an acute phase of short, intense health impairment followed by a failure to recover and a subsequent chronic phase of more prolonged, less intense impairment. Nevertheless, this concept of the level of illness or injury impacting the level of welfare compromise is helpful when evaluating the welfare of acute and chronic feedlot cattle.

4.3 Case study: applying the Five Domains Model

During a routine home pen check of healthy animals, a producer observed a steer presenting with open mouth breathing and coughing,

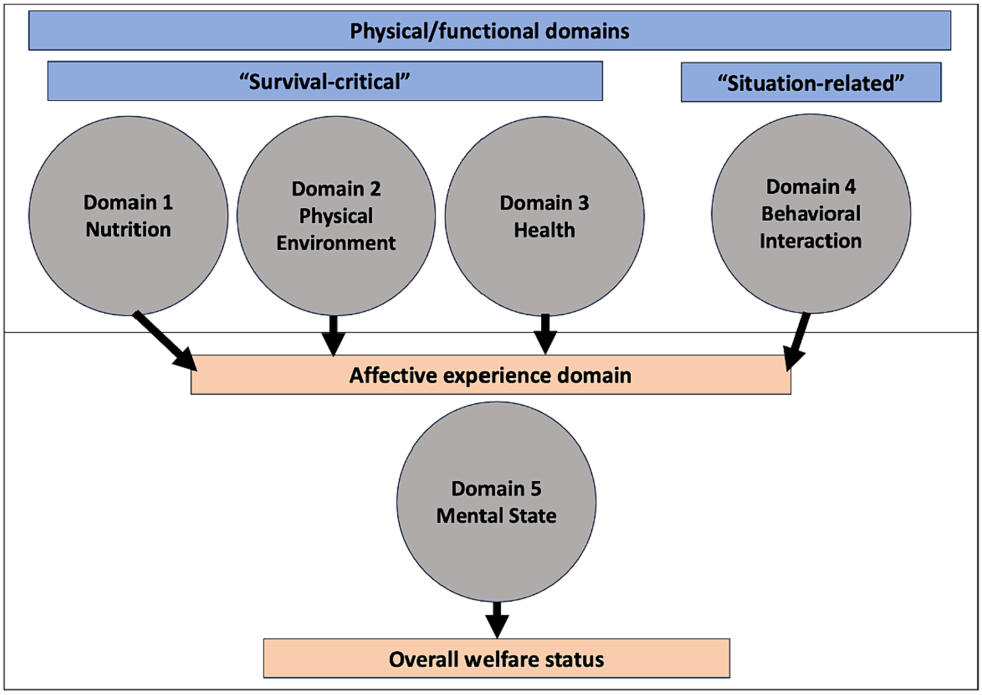

FIGURE 2
A diagram of the Five Domains Model of animal welfare. Adapted from Mellor et al. (20); licensed under CC BY 4.0.

a depressed attitude, and appetite loss (anorexia). The steer was flagged for further evaluation and walked to the treatment facility. After a temperature check and lung auscultation, the steer was diagnosed with acute BRDC and treated according to feedlot standard operating procedures (SOPs). Using Table 2, the producer then evaluated the steer's current welfare status to decide if the steer should be returned to his home pen or moved to a hospital pen. Firstly, it was noted that the steer was well-conditioned with a body condition score (BCS) of 6 (good) on a 9-point scale (96). During recent pen checks, the steer was observed at the feed bunk and waterer, and the rumen appears to have feed present (good gut fill). This indicates that despite the potential decrease in feed and water consumption common during sickness (56, 74, 81), the steer seemed to be consuming feed and water. Thus, the evidence indicated that the Nutrition Domain was relatively unaffected despite the BRDC diagnosis. At the time of evaluation, high ambient temperatures represented thermal extremes and an impact on the Physical Environment Domain. This interacted with the Health Domain, as cattle with BRDC may have impaired pulmonary capacity (97). Since cattle in high environmental temperatures thermoregulate via evaporative cooling (95), this may inhibit their ability to cool their body (98). The open-mouth breathing observed during the pen check (which was not observed in other steers in the pen) suggested that Physical Environment and Health Domains were impacted, and the associated mental effect of thermal discomfort, overheating, and breathlessness, which is considered a significant animal welfare issue (99), were impacting the steer's welfare. Other physical impairments stemming from the acute BRDC (Health Domain) were likely experienced through the Mental State Domain with negative mental affects such as lethargy and dullness, pain, and nausea. Finally, the steer was housed in a familiar home pen environment with a familiar social group and did not seem to be isolating itself from the group. This provided opportunities for positive mental affects such as affectionate sociability from familiar pen mates, and comfort and safety within a familiar environment. There was also no evidence of increased aggressive or agonistic behaviors in the pen or directed towards the ill steer, indicating that the increase in bullying or competition sometimes seen in ill animals was not occurring. Additionally, the physical environment of the home pen included well-maintained dirt mounds that provided a dry resting place and windbreak, which allowed the steer some level of agency in his ability to choose where in the pen he could best convalesce. Thus, there was no indication of negative mental affects in the Behavior Domain. However, the steer's agency could be further advanced with additional food resources and a shade structure that would provide additional choices for needs during convalescence. Overall, the primary sources of negative mental affects stemmed from the Health Domain and Physical Environment Domain, and there were sources of positive mental affects stemming from the Behavior and Nutrition Domains. Since the negative mental affects were related to the BRDC-related clinical signs, and the positive mental affects from the location of the steer in his home pen, the producer decided it was in the best interest of the steer's welfare to keep him in his home pen instead of moving him to a hospital pen. Thus, the steer was returned to his home pen after treatment.

After a period indicated by feedlot SOPs, the steer was evaluated a second time to determine treatment success. Visual examination of the steer showed that clinical signs of BRDC were not improved. Additionally, the steer had a BCS of 5 (moderate), indicating it has lost some weight since treatment. Using Table 2, the producer noted that the main area of change from the previous evaluation was in the Nutrition Domain, with the decreased BCS indicating the steer may have been experiencing intermittent hunger (a negative mental affect).

As this was the only noticeable change from the previous evaluation, and the steer still seemed to be able to move freely within his home pen's physical and social environment without difficulty, the producer decided to give a second treatment and return the steer to his home pen.

At the third evaluation, it was clear that the steer was still not responding to treatment and had experienced a significant decline since the last treatment. The steer now had a BCS of 3 (thin), a visibly concave rumen (no gut fill), and a visibly sunken and dry orbital area, indicating he had not been eating or drinking enough to maintain body weight and hydration. Thus, he was experiencing moderate to severe negative mental affects (Mental State Domain) via hunger, dehydration, and potentially weakness from starvation associated with the Nutrition Domain. The steer was observed open mouth breathing even during early morning pen checks before ambient temperatures were high. This indicated potentially significant impairment in pulmonary capacity (Health Domain). Combined with a lack of shade structures in the pen to protect from thermal extremes (Physical Environment Domain) and the inability of the steer to exercise his agency by seeking these shade structures as needed (Behavior Domain), the steer was likely experiencing significant negative mental affect due to breathlessness, overheating, and helplessness from the inability to seek shade or other methods of thermal regulation. These negative mental affects likely outweigh the potential positive affects the steer was experiencing from the familiarity of his home pen and social environment. This third evaluation of the steer's welfare using Table 2 led the producer to conclude that the current resources provided to the steer in the home pen were insufficient for him to recover or maintain his welfare during illness and that action needed to be taken. Thus, the producer decided to treat a third time and move the steer to the chronic pen for closer monitoring, and where additional resources such as shade, long-stem hay, corn-stalk bedding, and additional floor space were available.

At the fourth evaluation after being moved to the chronic pen, the steer seemed to be potentially on the road to recovery. Since the chronic pen had fewer cattle and was checked twice as often as the home pens, the producer noted that the steer had been spending much of his time either eating from the long-stem hay feeder or resting under the shade structure in the pen. His body condition score had improved slightly (BCS 4, moderate), and there was evidence of gut fill, indicating he had recently eaten. Thus, the Nutrition Domain was improved, and he was likely experiencing the positive mental effect of satiety. The physical environment of the chronic pen, which included additional bedding and shade structures, represented an improvement in the Physical Environment Domain via effective shelter and shade, and the steer could experience improved thermal comfort from utilizing these resources. His ability to exercise agency and make choices (Behavioral Interaction Domain) was improved through the increase in resources available in the pen (shade, hay, bedding, etc.), which provided the steer with opportunities to experience positive mental affects such as confidence and feeling in control. One potential risk from the move to the chronic pen was the change in physical and social environment, which could have prompted negative mental affects such as neophobia, anxiety, loneliness, and insecurity from the unfamiliar pen and pen-mates. Fortunately, there was no evidence of this, as the steer was observed integrating into the chronic pen well with no evidence of isolation, bullying, or competition for resources. Thus, overall the steer has overall positive changes in his welfare state indicated by the Five Domains. At this point, the producer will continue monitoring the steer to ensure his recovery continues, so they can make further interventions down the road if needed. It is important to note that while the steer recovered after moving to the chronic pen in this case, there are cases where this will not occur, and the animal may continue to decline. In that case, the information in Table 2 can serve as a guide for timely euthanasia decision-making. Producers should consider the balance between positive and negative mental affects, the length and severity of suffering, and the likelihood of recovery, and how these impact the animals' overall quality of life.

5 Discussion

The aim of this review was to provide a comprehensive overview of the acquired knowledge regarding ill and injured feedlot cattle welfare, focusing on existing knowledge gaps and implications for hospital and chronic pen management and welfare assurance. During the preliminary literature search, 110 papers with mention of ill or injured feedlot cattle welfare were identified. While this number of papers at first seems to indicate that this has been a well-studied subject, a closer look shows that many of these papers made only one or two mentions of cattle welfare. Similarly, many papers were conducted on a healthy population and collected one or two health outcomes (such as morbidity or BRDC incidence). Thus, while these papers were flagged based on the literature search terms, the study's primary goal or population of interest was not ill or injured feedlot cattle or cattle welfare. This suggests an opportunity to purposefully integrate animal welfare outcomes into study design, particularly for studies that include ill and injured feedlot cattle as the population of interest. The second and third literature searches, which focused on managing ill and injured feedlot cattle, resulted in only 12 papers that mentioned hospital-type pens and two that mentioned chronic pens. Hence, published research with direct implications for managing this vulnerable population to maintain their welfare is scarce. The preponderance of studies relating to BRDC was unsurprising, as it remains the primary cause of morbidity and mortality in U.S. feedlots, and some cattle populations do not respond to treatment (100, 101). Other less common etiologies such as lameness, digestive issues (e.g., bloat, acidosis), and buller-steer syndrome are also important for ill and injured cattle welfare, and regional and housing-related differences across feedlots can impact the prevalence of different etiologies in their cattle. For example, the prevalence of digital dermatitis (a lameness-causing disease) in cattle herds varies across operations (102, 103) and housing conditions (102, 104). Lameness is the most common reason for an animal to be railed (31), which indicates that it is an important condition to consider when dealing with chronic animal populations. Lameness is associated with pain and discomfort (105), and bloat is also a painful condition (106), which has direct impacts on animal welfare, especially given that pain mitigation is not always consistently given to ill or injured cattle in feedlots (24).

There is a need for more focused research on specific subpopulations of ill and injured cattle, to provide a sound foundation of knowledge that can be referred to create benchmarks for audits and welfare assurance programs. Chronic cattle populations have received the least research attention, and they can vary greatly from feedlot to feedlot, both in total number, diagnosis, and final dispositions, all of which can have

TABLE 2 Application of the 2020 Five Domains Model (20) to evaluate the welfare of ill or injured feedlot cattle[1].

Domain	Condition(s)[2]	Outcomes indicating positive welfare	Associated positive mental affects (Mental State Domain)	Outcomes indicating negative welfare	Associated negative mental affects (Mental State Domain)
Nutritional – "The water and food available to animals"	Water intake: (−) restricted (+) correct quantities	Presence at waterer; signs of good hydration status	Pleasures of drinking (quenching)	Dehydration [e.g. sunken eyes, dry mucous membranes (93)], absence from waterer; competition at waterer	Thirst; weakness from dehydration
	Feed intake: (−) restricted (+) correct quantities	Good body condition score; good gut fill (full rumen); presence at the bunk during feeding events	Satiety	Poor body condition score; no gut fill (empty rumen); competition at the bunk; absence from bunk during feeding events	Hunger; weakness from starvation
	Food variety & quality: (−) poor quality, low variety (+) high quality, high variety	Good body condition score; use of alternative sources of food (e.g., hay)	Pleasures of food tastes/ smells/textures; masticatory pleasures	Poor body condition score; absence or lack of use of alternative food sources	Hunger; malaise from malnutrition; eating-related boredom
Physical Environment– "The impacts of physical and atmospheric conditions to which animals are exposed directly"	Pen flooring: (−) uncomfortable, unclean (+) comfortable, well maintained	Good mud score (18); ease of postural changes	Physical comfort, thermal comfort	Poor mud score (18); physical evidence of skin irritation; pain behaviors when moving or lying	Physical discomfort: musculoskeletal pain, skin irritation, difficulty of movement
	Thermal environment (−) thermal extremes (+) effective shelter and shade	Signs of thermal comfort, use of available shelter and shade resources	Thermal comfort	Signs of overheating [open mouth breathing, high respiration rate; (94)] or chilling [shivering, huddling; (95)]	Thermal discomfort: chilling, dampness, overheating
Health – "The impacts of injury, disease and different levels of physical fitness"	Injury (acute, chronic, husbandry mutilations): (−) present (+) absent	Absence of physical signs in injury	Comfort of good health and functional capacity	Physical signs of injury (presence of cuts or lacerations, lameness)	Pain (many types), breathlessness, debility, weakness, sickness, malaise, nausea, dizziness
	Illness (acute, chronic): (−) present (+) absent	Absence of clinical signs of disease		Clinical signs of disease (temperature, nasal discharge, depressed temperament, etc.)	
	Functional impairment (e.g. amputation, genetic, lung, heart, kidney, neural): (−) present (+) absent	Absence of functional impairment		Presence of functional impairment (may be the result of a previously resolved illness or injury)	
Behavioral Interactions – "Interactions with humans, the environment, and other animals"	Agency and interaction with the environment:				
	Environment-focused activity: (−) present (+) absent	Behavior (e.g. normal activity, utilizing/exploring pen space)	Interest, pleasant occupation; calm, in control; engaged by activity, focused	Behavior (e.g. low activity, not utilizing/exploring pen space)	Various combinations: startled by unexpected events, neophobia, hypervigilance, anger, frustration, negative cognitive bias
	Foraging opportunities (−) present (+) absent	Behavior (e.g. bunk use, exploration of the pen, use of alternative food sources)		Behavior (e.g. bunk use, exploration of the pen, lack of or disuse of alternative food sources)	
	Agency and interaction with other animals:				
	Significant threats and limits on threat avoidance, escape, or defensive activity: (−) present (+) absent	Behavior (e.g. low levels of agonistic or aggressive behaviors; opportunities for escape and use of refuges; no limitations on sleep/rest)	Secure, protected, confident	Behavior (e.g. presence of agonistic or aggressive behaviors); physical signs of targeted bullying (e.g., buller animals)	Anger, anxiety, fear, panic, insecurity, neophobia
	Animal-to-animal interactive activity (−) present, positive (+) absent, negative	Behavior (e.g., allogrooming, proximity to known conspecifics, other positive affiliative behaviors)	Affectionate sociability	Behavior (e.g., isolation, decreased positive social interactions and play)	Loneliness, depression, yearning for company; thwarted desire to play
	Agency and interaction with humans:				
	Animal handling (−) poor (+) good, utilizes low-stress handling methods	Human behavior (e.g., patient, gentle, quiet, confident, kind, empathetic, subtle pressure cues); cattle behavior (e.g., short flight distance, calm alertness, compliantly responsive, seeks contact).	Calm, confident, at east, feels in control; enjoys variety	Human behavior (e.g., impatient, shouting, uncertain, fearful, indifferent, harsh pressure cues); cattle behavior (e.g., long flight distance, hypervigilant, attack/fight, escape, avoidance, freezing, non-compliant)	Anxiety, fear, panic terror, neophobia; insecurity, confusion, uncertainty, persistent, unease; helplessness; pain from injuries; negative cognitive bias
	Caretaker aptitude: (−) inexperienced, untrained, unskilled (+) trained, experienced, skilled				

[1]The conditions and associated mental affects presented in this table do not represent a comprehensive list of all positive and negative welfare indicators, and no single outcome is a conclusive indicator of welfare state. This table should be applied on an individual animal, case-by-case basis, with careful consideration of how the listed (and un-listed) outcomes combine in a multi-modal approach to welfare assessment.

[2]Negative conditions are preceded by (−); positive conditions are preceded by (+).

implications for chronic cattle welfare. One descriptive epidemiological study for calves entering a central Saskatchewan feedlot in the Fall of 1998 reported that 1.3% (158 calves) become chronic cattle (27). The 2011 NAHMS data indicates that the prevalence of chronic cattle treated for respiratory disease may be as high as 3.6% (24). Updated epidemiologic data on ill and injured cattle populations in commercial feedlots, especially chronic cattle, would help characterize these populations and potential risk factors for future research.

Blakebrough-Hall et al. investigated the effects of BRDC on economic outcomes and concluded that as the number of BRDC treatments increased from 0 to ≥3, feed costs and total value at slaughter decreased linearly. Additionally, cattle treated ≥3 times for BRDC grew 0.7 kg/d less and had carcasses 39.6 kg lighter than cattle never treated for BRDC (107). These findings have implications for the economic impacts of chronic cattle, as receiving ≥3 treatments is a common definition for chronically ill cattle. During an assessment of chronic pens at five Iowa beef feedlots, it was estimated that costs associated with treating cattle with chronic BRDC can range from 85 to 105 USD, and for chronically lame cattle mean treatment costs were around 63.48 USD. Additionally, there was an average daily maintenance cost of approximately 6.80 USD per head per day in the chronic pen, and chronic BRDC cattle with mortality outcomes had an average net profit of −946.50 USD (108, 109). Thus, there is evidence that certain management decisions, such as the amount of time cattle spend in the chronic pen and incur a daily maintenance cost, can impact the economic returns of an individual chronic animal. Economic data can also help with euthanasia decision-making, as managing cull animals in the feedlot is an essential part of a marketing strategy that optimizes feeder cattle health, welfare, and performance while minimizing death and economic losses (29). Thus, developing and implementing evidence-based guidelines for managing ill and injured feedlot cattle could help strengthen both cattle welfare and economic outcomes in chronic pens by helping feedlots manage their chronic pens in a way that balances these two important outcomes.

There is also a need for a clearer understanding of the behavioral responses of ill and injured feedlot cattle, how these behaviors vary with etiology and disease severity, and the implications of these behavioral variations for cattle management and welfare. Most sickness behavior studies of feedlot cattle focus on cattle with respiratory disease, many with the goal of early detection of morbid cattle using behavioral changes (110) and technological tools (86, 111). While early disease identification is vital for implementing effective BRDC therapeutics (112) and various lameness conditions (113), greater scrutiny of sickness motivation is needed to better understand the trajectory of convalescence and recovery, together with associated opportunities to improve pen designs. For example, does adding additional food resources to a chronic pen (such as long-stem hay) benefit all chronic cattle or only cattle with certain etiologies? Do cattle with chronic BRDC benefit from heat-mitigating resources such as shade, misters, or sprinklers, and which of these is the most beneficial and economically viable to implement? There is also evidence that sickness behavior expression differs for cattle with differing severity of parasitic infections (82–84). However, no research has been done on if this is true for other diseases and injuries. Since disease severity can vary greatly in other illnesses and injuries besides parasitism and for acute vs. chronic cattle, this may have implications for the identification, management, and welfare of these cattle. Finally, additional areas of research such as pen design (e.g., shade, wind breaks, space, flooring, nutrition, commingling) and diagnostics and health protocols (e.g., diagnostic tools, precision livestock technologies, animal record management) should be investigated and validated in field-based settings to help understand the short- and long-term effects on promoting ill and injured cattle convalescence, recovery, and welfare. As this additional research leads to the development evidence-based guidelines for ill and injured cattle management, collaboration with industry stakeholders and feedlot professionals will be vital to successfully implement and refine guidelines to ensure they are practical and effective in commercial feedlot settings.

To the authors' knowledge, this review represents the first time that the Five Domains Model has been applied as a framework to evaluate the welfare of ill and injured feedlot cattle. It is important to recognize that every feedlot is different and may have different needs and possible solutions that work for their operation. Short case studies documenting what has (and has not) worked for individual feedlots to manage their ill and injured cattle populations would add valuable information to the knowledge base. This is especially true for managing illnesses and injuries that are less reported than lameness and BRDC—such as blind cattle, cattle with digestive issues, and cattle with neurological issues. In addition, there are opportunities for research on management factors that are involved in managing ill and injured cattle populations, such as producer training, economics, and records, which may reveal synergies between animal care and feedlot operation productivity. Finally, the Five Domains Model has been used before to help develop welfare assessment guidelines (114), and this approach could also be used to aid in the development and improvement of feedlot audits and welfare assurance schemes that can properly assess feedlots on their management of ill and injured cattle.

Dissemination of knowledge gained to current and future veterinarians, producers, and feedlot personnel is vital to ensure meaningful improvements in chronically ill or injured feedlot personnel management and welfare. Ensuring that information is provided in an accessible format is vital. Hands-on learning experiences have been shown to be the preferred method of instruction for cattlemen (115). In a 2014 survey, feedlot managers reported that most of their information on lameness prevention came from feedlot veterinarians, nutritionists, and training seminars (116). Feedlot nutritionists indicated that peer-reviewed journals were of great importance in their information-seeking behaviors (117). Ensuring that key subjects pertaining to ill and injured feedlot cattle management and welfare are a part of the veterinary curriculum is also important. In a 2021 survey of 10 U.S. veterinary schools, the authors concluded that veterinary schools should consider incorporating more advanced euthanasia training programs into curriculum (118). A 2021 survey of representatives from eight veterinary schools in Australia concluded that while most universities covered relevant materials using a variety of methods, at two schools that relied solely on clinical cases not all students will be exposed to making euthanasia decisions (119). Literature suggests that flipped classrooms (120), hybrid learning (121), and competency-based approaches (122, 123) are all promising teaching strategies that could enhance veterinary student learning. Finally, Terrell et al. found that around 11% of feedlot managers used internet-based sources for information (116); social media may prove a valuable resource for teaching and engagement in agriculture topics in the near future (124). Ultimately, as research and knowledge generation on the important topic of ill and injured cattle management

and welfare continues to grow, it is vital that dissemination of knowledge to and collaboration with current and future professionals in the feedlot industry is emphasized to maximize the positive impacts on cattle welfare.

6 Conclusion

In this literature review articles on the management and welfare of ill and injured feedlot cattle were identified. Most articles relating to ill and injured feedlot cattle welfare were conducted on a healthy population with one or two measured health outcomes, indicating that there is a need for studies focusing on ill and injured feedlot cattle as the population of interest. The even greater sparsity of papers on managing ill and injured feedlot cattle in specialized hospital or chronic pens further suggests that there is a need for published research with direct implications for managing this vulnerable population to maintain their welfare. BRDC is by far the most prevalent diagnosis for acutely ill feedlot cattle, and a small percent of these cattle will become chronically ill. While other diagnoses, such as lameness, digestive issues, and pneumonia, are less prevalent, they also have important implications for cattle welfare. Research is needed to better understand these conditions and their welfare impacts. Cattle with varying diagnoses and severity of conditions will display similar behaviors during convalescence, and these behavioral responses can be used to design facilities that accommodate cattle convalescent behavioral needs. Additional research is needed to provide evidence-based best practices for hospital and chronic pen design and management. Proper application of the Five Domains Model to individual cases can help producers identify impaired cattles' feelings and experiences and subsequent welfare outcomes to aid in management decision-making and pen design. Ultimately, by outlining the current knowledge of ill and injured feedlot cattle and utilizing this knowledge to assess cattle welfare, this review provided an essential step towards the ultimate goal of strengthening the care of ill and injured feedlot cattle.

Author contributions

ES: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. GD: Conceptualization, Funding acquisition, Project administration, Writing – review & editing. RD: Conceptualization, Funding acquisition, Writing – review & editing. AJ: Conceptualization, Writing – review & editing. DT: Conceptualization, Writing – review & editing. SM: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This project was supported by the United States Department of Agriculture and National Institute of Food and Agriculture, grant #2021–67015–34080.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Veterinary Science

TYPE Mini Review
PUBLISHED 30 May 2024
DOI 10.3389/fvets.2024.1394113

OPEN ACCESS

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RECEIVED 13 March 2024
ACCEPTED 29 April 2024
PUBLISHED 30 May 2024

CITATION
Neves MM, Klein SG, Silva RC, Bernardes LMM, Malta SM, Vieira TN, Rosa RB, Lima IL, Ferreira FB and Silva MV (2024) Impact of quality and transparency in scientific writing on the reduction of animal usage in experimental protocols: a review based in pertinent literature.
Front. Vet. Sci. 11:1394113.
doi: 10.3389/fvets.2024.1394113

Impact of quality and transparency in scientific writing on the reduction of animal usage in experimental protocols: a review based in pertinent literature

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The irreproducibility in scientific research has become a critical issue. Despite the essential role of rigorous methodology in constructing a scientific article, more than half of publications, on average, are considered non-reproducible. The implications of this irreproducibility extend to reliability problems, hindering progress in technological production and resulting in substantial financial losses. In the context of laboratory animal research, this work emphasizes the importance of choosing an appropriate experimental model within the 3R's principle (Refine, Reduce, Replace). This study specifically addresses a deficiency in data specification in scientific articles, revealing inadequacies in the description of crucial details, such as environmental conditions, diet, and experimental procedures. For this purpose, 124 articles from journals with relevant impact factors were analyzed, conducting a survey of data considered important for the reproducibility of studies. Important flaws in the presentation of data were identified in most of the articles evaluated. The results of this study highlight the need to improve the description of essential information, standardizing studies, and ensuring the reproducibility of experiments in areas such as metabolism, immunity, hormones, stress, among others, to enhance the reliability and reproduction of experimental results, aligning with international guidelines such as ARRIVE and PREPARE.

KEYWORDS

laboratory animal science, data reproducibility, 3R's, scientific writing, animal models

1 Introduction

The construction of scientific thought involves the necessity of a methodology, which must be well-structured in the written text so that the new knowledge generated can be reliable and well-founded, and, if necessary, can be revised as a way of generating even more information (1). Therefore, under the stipulated conditions in the construction of a scientific article and

using the appropriate methods, it is possible to arrive at the same results and conclusions as those that were previously done and shared.

However, this is not the reality of contemporary research, according to a survey conducted by the journal Nature (2), which considered the experiences of more than 1,500 researchers. On average, more than half of the publications are not reproducible under the described methodology. Thus, the foundations for the trust and accuracy of the results produced in the light of scientific knowledge are often called into question, due to confusion and a lack of guidance in scientific writing.

In this context, irreproducibility in different areas of science causes a series of problems, ranging from the unreliability of the produced results, which hinders progress in technological production in various areas, to enormous financial expenditure without justifiable returns. In the biomedical research field, where experimental animal models are crucial, no different information is expected. For example, an annual loss of 28 billion dollars in biomedical research without fruitful outcomes due to this irreproducibility (3).

When considering the life sciences, research on drugs, treatments for diseases, and understanding pathologies, the confidence in the results is essential for the advancement of the quality of life and health of humanity. However, like other areas, there are various obstacles to reproducibility which becomes even more significant when considering the use of animals as experimental models (4). This reality can occur due to various factors, such as bias in research construction, as well as problems in writing and data selection (5) and poor experimental design (6–9).

In the scope of animal research, the choice of an appropriate experimental model ensures an important step in the experimental design of studies that seek to understand the physiological, anatomical, and genetic functioning of a specific topic in relation to what occurs on a biological scale and can possibly be extended to the human context (5). Furthermore, it is important that experimental protocols are well described in works published in scientific journals. This practice significantly impacts the reproducibility of data, as it standardizes protocols and makes documentation on these procedures more robust (2) It has already been raised that the omission of data has contributed to the reproducibility crisis (3, 10).

Within the framework of the 3R principle (Refine, Reduce, Replace) proposed by Russell and Burch, experiments involving animals should be replaced with alternatives whenever possible. However, if an animal experiment cannot be replaced, the number of animals should be limited, and procedures should be refined to minimize the pain, suffering, and distress caused to the animals by the experiment (11).

Considering the data deficiency situation in scientific articles, our group has been seeking to understand the reasons behind such a crisis. This discussion proves to be of great importance for the improvement of science because it will present a reflection on how researchers sometimes may contribute to this reproducibility crisis. Not just because the experiments are flawed, but at the time of writing the article data, they do so inadequately. Our results can lead to positive outcomes, such as the non-use of animals when not necessary, as there is already access to previously described data That said, this present work aims to analyze how scientific articles report relevant information involving animal models and how their description can contribute to limited reproducibility. For this purpose, 124 articles from different journals with considered relevant impact factors were evaluated. Data presented in each article, such as approval by the Institutional Animal Care and Use Committee (CEUA), brand of feed, environmental factors, anesthesia, analgesia, and statistical data, were surveyed.

In this review, we synthesize information from various studies pertinent to laboratory animal science, discussing the main topics that must be considered in the writing and planning of a scientific paper inserted in these subject areas in order to make it robust, reliable, reproducible, and to provide an ethical scientific context that adheres to recommendations for animal care and experimentation, prioritizing the health of both the animals and the researcher.

Within the literature, significant studies showcase how the composition of a scientific article influences scientific reproducibility. Therefore, as a distinctive feature, we aim to illustrate how the comprehensive description of the model and its associated aspects impact not only reproducibility but also the ethics of animal usage.

Accordingly, our review aims to address essential aspects in the science of laboratory animal research and how their detailed description in studies can contribute to more reliable science.

2 Methodology

The paper selection for this review followed stringent criteria to ensure the inclusion of relevant and impactful studies. Specifically, peer reviewed and relevant in the biomedical field, published between 2015 and 2020 with a journal impact factor surpassing 1.0 were considered. The impact factor calculation used a chrome extension named "PubMed Impact Factor" and considered the Journal Citation Reports (JCR) Quartile, with inclusion limited to papers falling within Quartiles Q1 to Q4. Inclusion criteria also involved the presence of the keyword "animal model" and "mice" and "experimental models" to focus on studies directly related to animal models. English-language papers were exclusively chosen, and the selection prioritized studies involving animals other than humans. To maintain thematic integrity, review papers were excluded, as were studies that did not incorporate animal models.

The selection was made based on the following research framework in PubMed database: "(("2015/01/01" [Date - Publication]: "((("2015/01/01"[Date - Publication]: "2020/01 / 01"[Date - Publication])) AND (animal model[Text Word])) AND (mice[Text Word])" and an Impact Factor filter. In this research, 250 articles were randomly selected for analysis, and upon applying the inclusion and exclusion criteria, 124 articles remained.

The articles we consider encompass the following experimental models: mouse, rat, *Drosophila melanogaster*, *Aelosoma viride*, Eurasian blue tit, cattle, dragon lizard, *Lampronycteris brachyotis*, *Micronycteris megalotis*, *M. microtis*, *M. homezi*, *M. minuta*, and rabbit. All the studies used are from the biomedical field or related to biological sciences.

3 The contribution of transparency in article writing to the ethical use of animals

As described above a total of 124 articles from various journals, with a considered significant impact factor (above 1.0), were examined (articles available at Supplementary information S1). An analysis of the data presented in each article was conducted, encompassing

presence of statistical methods for group formation, the brand of feed used, environmental variables, anesthesia, and analgesia procedures, as well as statistical data.

The results revealed a considerable number of factors with unsatisfactory information, significantly contributing to the lack of reproducibility in assays. For instance, only 12.9% of the articles mention the brand of food used. Over half of the articles fail to specify crucial details, such as brightness, temperature, and humidity to which the animals were subjected. Additionally, 60.5% of the articles do not describe the euthanasia method employed, and 91.1% do not present the calculation formula of the sample size, among other elements that can directly impact the reproducibility of the research (Figure 1).

Handling laboratory animals requires rigorous data control. All the data presented in this study are directly related to the health, well-being, and immunity of the animals. Depending on the study to be conducted, this data becomes extremely important. Any changes in the photoperiod can lead to hormonal alterations (12), impacting the results. Nutritional or environmental changes can alter factors related to immunity (13), once again causing a negative impact on the reproducibility of desired results.

Thus, by highlighting these shortcomings in data specification, the need to improve the description of essential information (diet, environmental factors, pain-related factors, among others) becomes evident. With the deficit of these details, experiments that would not need to be repeated may be conducted again, contradicting the principles proposed by the 3R's (14) and the sustainable development goals outlined by the UN (15).

To prevent this, scientific journals and platforms should require the complete disclosure of data from experimental models, preferably following international regulations such as the ARRIVE guideline or the PREPARE guideline (16).

The goals of sustainable development are linked to animal welfare. Animals in a state of well-being are more productive and yield products of higher quality. Similarly, research animals generate more reliable results and foster more promising technologies and innovations. Furthermore, more effective public policies from veterinary bodies and responsible entities can guide and implement positive actions for animal welfare (15).

To this end, there are some published guidelines, such as ARRIVE and PREPARE, which can contribute to animal welfare and the refinement of research. The guidelines encourage researchers to report on randomization, blinding, sample size calculations, management and procedures, welfare monitoring, euthanasia, among others. Thus, under firm convictions about the importance of these issues and supported by evidence from other areas of research, there is a current consensus that scientists should adopt these practices whenever possible to produce work with greater impact and applicability (16).

4 The importance of experimental models for the development of science, technology, and innovation

Vertebrate animals have been used as models of anatomy and physiology since the beginning, where there are records of Greek doctors who dissected animals for anatomical studies (17). In the 17th century, the moral questions surrounding the use of animals began to be raised and between the 19th and 20th centuries, the pharmacopoeia included effective and scientifically tested medicines, which led to a greater understanding of the importance and validity of animal-based research (18, 19).

Currently, animal models are essential for several fields within biomedical research, such as cancer, neuroscience, pharmacology and toxicology, neurobiology of diseases, endocrinology, public health, palliative medicine, discovery and testing of new medicines, vaccines and other biological products whose validation requires preclinical animal studies (19). Its use is based on the principle of replicating physiological and pathological processes, with the species selected according to the objective and hypothesis of each project (20).

In recent years, for example, different animal species have been used to study the 2019 Coronavirus pandemic. Through murine, primate, porcine and even zebrafish models, neurological, behavioral, cardiovascular, and oncological disorders can be studied as they are also new therapeutic approaches are being developed. Recently, nematodes and arthropods are some of the new alternatives (21). Today, the majority of species used in biomedical research are rodents, as they are considered ideal models for studying pathologies that affect human populations due to their physiological homology (21).

We can reflect the importance of using animals by observing the number of important studies, such as those for the Nobel Prize in Physiology and Medicine, in which 90% of them used animals (22). In 2005, a global survey was carried out, estimating the number of animals used (23). This estimate showed the use of 58.3 million animals in 179 countries. This same group made an estimate for 2015, which was around 79.9 million animals, an increase of 36.9% compared to 2005 (24). The Mutual Society (not-for-profit organization) "Understanding Animal Research" has data from 2020 and recorded that the European Union used 8,624,692 animals, with 91% of the animals used for experimental purposes being mice, fish, rats, and birds, while cats, dogs and primates represented 0.2%. In the USA, unofficial estimates that include mice, rats and non-mammalian vertebrates estimated the use of 12 to 24 million animals. In Canada, the Canadian Council on Animal Care (CCAC) reported that 5,067,778 animals were used in 2020. Therefore, we can conclude that many studies around the world use animals, reaffirming their importance in science (25).

Currently, mice are the most used in human biology research (26), among all animals used in research, mice account for almost 60% of the total (27). This is due to their genetic and physiological similarity with humans, short gestation times, genetically homologous inbred strains, easy handling and easy maintenance (26).

5 Ethical and legal aspects in the use of animals and 3R's headings

Animal experimentation has, for an extended duration, been surrounded by a series of inquiries, both from the scientists conducting it and from the general population, questioning the obtained results at the expense of animal lives. In this context, an array of thoughts concerning animal well-being, ethics, and care have evolved over time. Presently, all these concepts are grounded in a set of three principles established by Russell and Burch in 1959 (11).

The guidelines are founded on the concepts of Reduction, Refinement, and Replacement, which establish ethical and legal

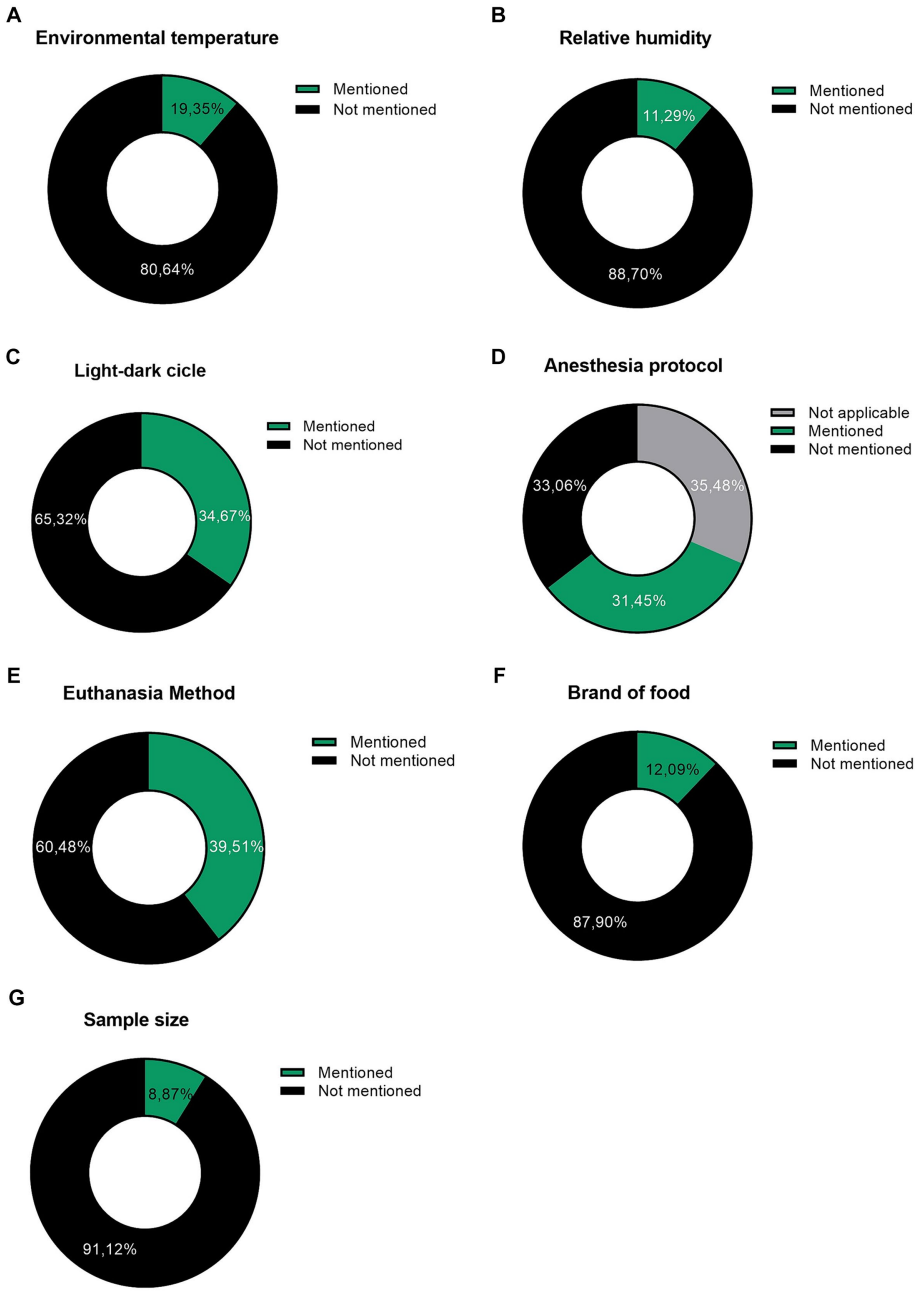

FIGURE 1
Graphs representing the items evaluated in the study articles. Environmental factors in animal facilities: **(A)** Environmental temperature. **(B)** Air humidity and **(C)** Light–dark cycle; Experimental procedures: **(D)** Anesthesia protocol and **(E)** Euthanasia method; Food: **(F)** Brand of food. **(G)**: Sample size calculation formula. The articles were evaluated for the presence of information, whether it was mentioned and whether it contained details. Despite being basic items, in most articles they are not mentioned. Data were expressed as a percentage, where 100% indicates a total of 124 articles.

concepts globally for the use of animals in re-search, imbuing a humanitarian perspective, dignity, and protection against suffering, pain, and stress upon these animals (1). It is vital to emphasize that, based on these principles, the entire legal and ethical framework of animal experimentation must be based on shaping the concept of what is dignified in the lives of animals, elevating them to a level of rights equivalent to humans. This principle, as advocated since 1973 and enshrined in the Swiss Constitution in 1992 and addressed by Bolliger ensures their moral standing (28).

In this context, several other countries have incorporated the idea of animals as beings with their consciousness, rights, and moral reality into their highest legislation, such as India, Brazil, Slovenia, Germany, Luxembourg, Austria, Egypt, in addition to the guidelines of the European Union and the United States. This shift in perspective considers animals as subjects with their rights and moral standing rather than mere property and objects, as they were often viewed (29). Consequently, limitations are set on how animal life can be manipulated and used in experiments, with specific barriers concerning sensations and intrinsic well-being guarantees.

It is worth emphasizing that the concept of dignity, and therefore the right not to suffer and to have one's needs met, which was once the exclusive domain of human beings, a concept upheld for a considerable period and reinforced by authors such as Giovanni Pico della Mirandola (30) and Immanuel Kant (31) has been extended to all animals. This is due to their sentience akin to that of humans, and their entitlement to the same rights, which ensures both ethical and legal legitimacy to the science produced with their assistance (32).

Furthermore, it is crucial to consider that research conducted without ethical considerations when it comes to animal use creates an experimental environment where the produced results cannot be relied upon. Ethical treatment implies the construction of well-being, ensuring the expression of phenotypes without alterations caused by stress (33). Thus, experimental reproducibility, a contemporary topic, especially when dealing with the use of model organisms, is only achievable when measures are taken, such as proper handling, anesthesia protocols, and stress avoidance, guaranteeing the veracity of the results attained in science (1).

It is important to emphasize that the new guidelines regarding the care of animal testing and experiments are primarily centered on those established in Directive 2010/63/EU by the European Union in 2010. This directive presented, suggested, and encouraged other legislations to adopt similar measures, a call heeded by countries across all continents. These regulations were constructed and based, once again, on the concept of dignity. The idea is that a legal and punitive framework can only be established for those who disregard it when there is a set of principles defining what is dignified and guaranteed for animal life. It asserts that the right of animals, being lives that should not be treated as mere possessions for utilitarian purposes, must be recognized. Instead, animals should be regarded as valuable contributors, with a significance equal to that of the researcher's existence, in the pursuit of scientific progress (34).

6 Important environmental factors in experimentation with rodents

Environmental factors are the set of variables that constitute the environment in which the animal lives, including physical, social and management aspects. In animal facilities, these factors are determined and monitored by man, since the environment is controlled. In this section we will emphasize the importance of detailing the housing and care factors of animals used in research to ensure experimental reproducibility, thereby facilitating a reduction in their use in subsequent studies. For illustration, we will exemplify physical factors, such as temperature, humidity, and luminosity (35).

Temperature is one of the first and most basic variables that must be observed when thinking about ambience. Animals housed outside their thermoneutral zone will have important physiological changes such as changes in metabolism, blood pressure, sleep, and rest time, circulating immune cells, among others (36). The most common in rat and mouse laboratories is housing below the thermoneutral zone, around 22°C, when the ideal would be around 30°C. This is mainly due to human thermal comfort, which is affected in these working conditions together with the use of personal protective equipment and activities carried out in animal facilities (36, 37).

To overcome this situation, some strategies can be used, such as maintaining an average temperature that does not affect animals or humans so much, associated with this, offering environmental enrichments that contribute to thermal insulation, such as materials for nesting and shelters and, whenever as possible, keep the animals in groups, so that they warm each other (38). In cases where the animal's thermoneutral temperature is lower than that of humans, environmental enrichment strategies with water, ice and ventilation may be useful.

The transparency in information regarding temperature is of paramount importance in the ability of a study to be reproducible without animal experimentation. This is because it allows for the standardization of a factor that directly influences animal behavior, in addition to their physiological and immunological functioning. This transparency enables the prediction of deviations in results across different repetitions due to variations in the temperature to which the animal is subjected.

As for relative air humidity, it is essential to guarantee the well-being and health of animals, as many species are sensitive to environmental variations. Humidity is directly related to thermal sensation as it can facilitate or impair gas exchange depending on the ambient temperature. In addition, air humidity much lower than recommended can lead to irritation of the airways and greater susceptibility to diseases such as Influenza (39). Therefore, careful monitoring and maintenance of air humidity in animal facilities is essential to ensure ideal breeding and experimentation conditions, promoting reliable and ethical results in scientific studies (35).

The same holds true for humidity in relation to temperature. When the humidity value is standardized between the reference work and the one being developed, the conditions, particularly pertaining to respiratory capacity and the animal's susceptibility to infections, become normalized. This is a crucial factor in some research studies, making it necessary to report these conditions in the animal housing section.

Light plays a significant role in animal experimentation, influencing both welfare and the scientific results obtained. Animals, such as laboratory rodents, are sensitive to light and dark cycles, and careful manipulation of these patterns is essential to maintain normal physiology and behavior (40). In addition to providing an adequate light source, regularity in light–dark cycles is crucial to preserving the animals' circadian rhythms, impacting important variables such as insulin resistance, gut microbiota dysregulation, sleep patterns and response to external stimuli (41). Controlled lighting also plays a role in minimizing stress in animals by promoting a more stable and predictable environment (42). Therefore, attention to luminosity is essential to ensure the validity and replicability of studies, while also considering the ethical impact and welfare of the animals involved in the experiment (43).

Finally, regarding the examples provided on how housing factors can alter the quality and reproducibility of an experiment involving animals, light also plays a significant role. It is essential to be transparent about this data because it not only influences factors such as normal behavior, reproductive capacity, microbiota, among others, but when presented excessively, it can cause direct harm to animal health, such as issues in the retina, hindering the faithful reproduction of a study (44).

Certainly, careful consideration of environmental factors such as temperature, humidity and light are essential to ensure reliable and ethical results in animal experimentation (43). It is worth noting that the information provided in this section is not the only important

aspect to consider in terms of transparency when writing a scientific paper and ensuring its reproducibility, especially in experiments involving animals. These examples are part of a much broader range that encompasses aspects such as analgesia and anesthesia methodologies, housing space and equipment, as well as statistical methods for forming groups with exclusion and inclusion criteria.

Other important criteria in the design of an experiment involving animal models should be guided, from its initial conception, by specific concepts and guidelines for reproducibility and experimental reliability. In this work, we will mention the ARRIVE and PREPARE guidelines. By recording these variables and understanding their direct influence on the well-being of laboratory animals, researchers can improve the validity and relevance of their studies. The search for conditions that mimic the animals' natural environment, combined with practices that promote their comfort, not only improves the integrity of experimental data, but also reinforces fundamental ethical principles (45) (see Figure 2).

7 ARRIVE and PREPARE guidelines

In contemporary times, with the advancement of biomedical research utilizing animals as experimental models, it has become essential to establish standards and guidelines for writing to emphasize key points for constructing transparent, comprehensible, and reproducible scientific papers. The first of these guidelines is known as ARRIVE (Animals in Research: Reporting *In Vivo* Experiments) (46). Proposed in 2010, it is directly related to a set of older recommendations from 1996, CONSORT (Consolidated Standards of Reporting Trials) (47). These guidelines were constructed based on systematic reviews and scientific research, along with input from animal experimentation experts worldwide. The goal was to identify, within the scientific scope, the necessary information to enhance the quality of work involving such models.

ARRIVE should not only be considered during experiments but throughout the entire thought, planning, and writing process related to the work. These aspects were well described in the original article suggesting this guideline, considering the quality of statistical planning and the presentation of data. It also suggests sharing details such as the physical characteristics of animal housing, demonstrating respect for animal dignity by the authors (46). Building upon ARRIVE, a set of enhancements was proposed in 2020, aiming to maximize the functionality and success of this directive. This resulted in the creation of ARRIVE 2.0, offering a more recent perspective and updating concepts derived from advances in scientific research. The objective is to increase adherence by both researchers and journals (48).

In this context, to complement the gaps addressed by ARRIVE and to synthesize the recommendations of the 2010/63 directive of the European Union (49), another set of guidelines was created: PREPARE

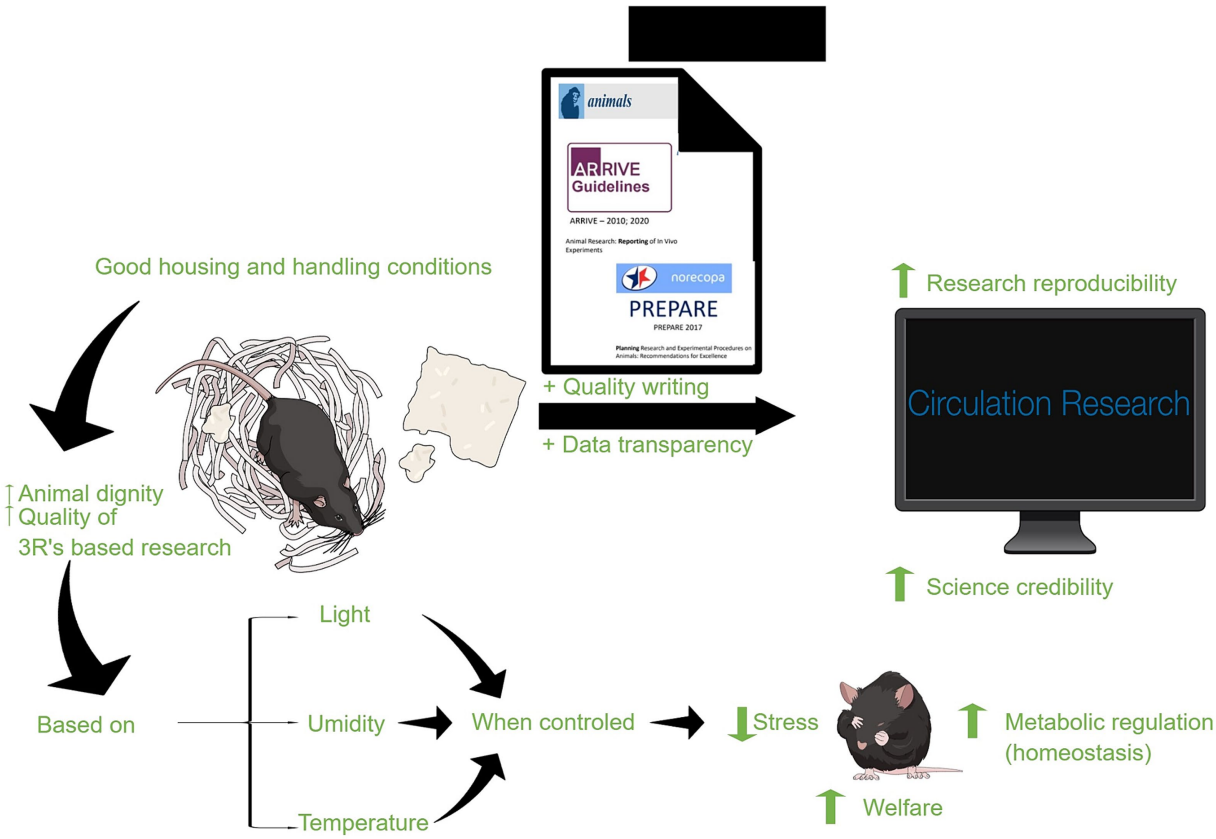

FIGURE 2
Factors that influence animal well-being and provide favorable conditions for their feeding, natural behavior, and self-care, leading to an increase in data confidence and reproducibility.

(Planning Research and Experimental Procedures on Animals: Recommendations for Excellence). Introduced in 2017, PREPARE is designed to be followed throughout the entire research and scientific writing process. It delves into ethics, proper care, respect for animal dignity, the researcher's relationship with the research institution, and animal care. PREPARE encourages transparency in presenting data and methodologies related to necropsies, sanitary and genetic monitoring, legal aspects, and detailed experimental procedures (50).

8 Discussion

The need to reduce the number of animals in research is based on several reasons of an ethical, scientific, economic, and social nature. In this paper we present data that point out ways to reduce the number of animals in experimental protocols at no cost, bringing up crucial points that are not presented in detail in many articles we evaluated. We show a reflection that will contribute to improving all the aspects presented above, and to improving the quality of life of professionals who work with laboratory animals. Currently, many professionals suffer from compassion fatigue, also known as emotional exhaustion, which is a psychological phenomenon that can affect veterinarians and laboratory animals technicians involved in animal experimentation due to constant contact with the suffering of animals (51).

In this article, we provide a specific insight into how the correct and transparent writing of a study involving research with laboratory animals serves as a means to share data that can lead to a reduction in the number of animals used. This is due to the possibility of faithfully reproducing experimental conditions, thereby eliminating the need for unnecessary repetitions and uses of large quantities of animals. Thus, in addition to all the other benefits already mentioned arising from quality writing, the major contributors to compassion fatigue, such as excessive euthanasia and continuous, repetitive exposure to protocols that induce animal welfare issues and desensitization to suffering, would be reduced and avoided (52). This fosters a healthier ecosystem for work and research.

The results obtained in this study highlight the importance of a thorough description of relevant data and information in articles. The need to address aspects such as environmental condition, pain control, and welfare improving methods. This approach not only contributes to the standardization of studies but also provides essential insights into metabolism, immunity, hormonal factors, and stress—fundamental components for ensuring the reproducibility of scientific assays, and to create a respectful and ethical research environment, both for researchers and for animals, these who are cornerstones for the advancement of science.

By emphasizing the significance of these elements, researchers can enhance the quality and reliability of their studies, fostering a more solid foundation for future research and scientific advancements. The inclusion of these critical details not only benefits the comprehensive understanding of experiments but also facilitates replication by other scientists, thereby strengthening the validity and robustness of the obtained results, in addition to reducing the need for unnecessary repetitions of experimental protocols and greater exposure to suffering and isolation by researchers, it decreases the possibility of compassion fatigue and prioritizes their mental health.

In summary, careful attention to specific aspects in the description of data in scientific articles not only addresses the demands of the academic community but also significantly contributes to the progression of science.

Author contributions

MN: Writing – review & editing. SK: Writing – original draft. RS: Writing – original draft. LB: Writing – original draft. SM: Writing – original draft. TV: Writing – original draft. RR: Writing – original draft. IL: Writing – original draft. FF: Writing – original draft. MS: Writing – review & editing, Writing – original draft.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This research was funded by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) – Rede Mineira de Biotecnologia em Modelos Experimentais (RMBME – FAPEMIG), pelo Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) e pelo Ministério Público do Trabalho de Uberlândia (MPT) and Universidade Federal de Uberlândia (UFU/PROPP).

Acknowledgments

The authors would like to thank Alessandra Moreira de Oliveira for her administrative and technical support at the Biotechnology in Experimental Models Laboratory – LABME and all technical team at Rodents Animal Facilities Complex, Federal University of Uberlândia. The authors would like to thank the editor for his guidance and assistance in structuring and organizing the content for the publication of this work, as well as the assigned reviewer for their insightful suggestions.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2024.1394113/full#supplementary-material

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frontiers | Frontiers in Veterinary Science

TYPE Systematic Review
PUBLISHED 11 July 2024
DOI 10.3389/fvets.2024.1429097

OPEN ACCESS

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RECEIVED 07 May 2024
ACCEPTED 18 June 2024
PUBLISHED 11 July 2024

CITATION
Linstädt J, Thöne-Reineke C and Merle R (2024) Animal-based welfare indicators for dairy cows and their validity and practicality: a systematic review of the existing literature.
Front. Vet. Sci. 11:1429097.
doi: 10.3389/fvets.2024.1429097

Animal-based welfare indicators for dairy cows and their validity and practicality: a systematic review of the existing literature

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Animal welfare is of increasing importance, with consumers preferring animal products made with ethical practices due to growing awareness. This shift highlights the need for reliable methods to evaluate welfare. This systematic review aims to assess the validity of current animal-based welfare indicators for dairy cows to aid farmers and agricultural professionals in evaluating and improving welfare amidst the lack of a clear legislative definition. The literature search spanned five databases: CAB Direct, PubMed, Scopus, Google Scholar and Livivo, covering publications in English and German from 2011 to 2021. Specific search terms were employed, and abstracts were screened for relevance. Publications were categorized based on exclusion criteria, with a final verification process conducted by three independent scientists. Research highlights correlations between welfare measures, farm characteristics and innovative indicators like hair cortisol concentration. Farming systems and housing methods significantly affect welfare, with pasture-based systems generally resulting in reduced lameness and improved behavior. Proper housing design and management practices are important, as they influence indicators like lameness and cleanliness. Heart rate variability and heart rate monitoring provide insights into dairy cow stress levels during milking and other stressors, making them valuable for welfare assessment. Biomarker research emphasizes the need to balance productivity and health in breeding strategies, as high milk production alone does not indicate good welfare. Behavioral studies and the human-animal relationship are key to understanding welfare. Precision Livestock Farming offers real-time assessment capabilities, although validation is needed. Stress physiology is complex, and while cortisol measurement methods are promising, further research is necessary. Assessment tools like the Animal Needs Index and routine herd data analysis are valuable for identifying welfare concerns. Key findings highlight the WQ® protocol's effectiveness and versatility, the challenge of its time demands, and the DCF protocol's promise for more practical and efficient welfare assessments. Commercial animal welfare audits should prioritize easily observable indicators and herd records due to logistical constraints in measuring biomarkers or heart rate variability. This focus on easily accessible indicators, such as body condition score, lameness, claw health, cleanliness, and somatic cell count allows effective welfare assessments, enabling prompt action to enhance wellbeing.

KEYWORDS

animal welfare, wellbeing, animal-based, dairy cow, welfare quality®, welfare indicators

1 Introduction

The subject of animal welfare is becoming more and more important in society (1). Public awareness is growing and the consumer is interested in products of animal origin which were produced under animal welfare-compliant conditions (1). With Article 13 of the Treaty on the Functioning of the European Union, the term welfare was mentioned in a European law for the first time in 2009. The animal is referred to as a "sentient being" whose welfare requirements are taken into account in political decisions of the EU and the member states (2). This gives rise to the problem that although the term animal welfare has made it into the EU treaty (2) it is not defined what exactly it is, despite the fact that it has such a big social relevance. Animal welfare is a critical issue, as it reflects societal values and ethical considerations regarding the treatment of animals. The inclusion of animal welfare in the EU treaty (2) signifies its importance at a policy level. The lack of a clear definition complicates the implementation and enforcement of consistent welfare standards across member states. Dairy cows often face unique welfare challenges, including issues related to housing, feeding, milking procedures, and overall health management. Despite their significant role in agriculture and the economy, the absence of tailored regulations leaves a gap in ensuring their well-being. This gap highlights the necessity for the EU to develop and enforce specific guidelines that address the welfare needs of dairy cows.

In Germany, animal protection has been a legally binding constitutional norm since 2002, when it was enshrined in Article 20a of the Basic Law for the Federal Republic of Germany (3). Article 1 of the German Animal Protection Law states that the well-being of animals as fellow creatures must be guaranteed (4), without a definition of the term being offered in this context.

Furthermore, Article 11 of the German Animal Protection Law stipulates that livestock owners must carry out internal checks to ensure that the requirements of Article 2 are met (5). For this purpose, "suitable animal-related characteristics (animal welfare indicators)" shall be collected and evaluated (6). The farmer must carry out a self-assessment regarding a not clearly defined animal welfare, with suitable indicators, which are not listed.

For a long time, it was believed that when an animal performs well (e.g., milk production), it feels comfortable (7). In other words, an animal that does not perform well does not feel well. In the meantime, it has been proven that there is a connection between production diseases in dairy cows and breeding with focus only on performance (genetic overload). This means that individual risk of disease (e.g., peripartum diseases) also has a genetic component and therefore, improved management and husbandry conditions cannot prevent all cases of disease (7). In addition to valid animal welfare indicators, other actions are also required, such as rethinking breeding targets in livestock husbandry. Less diseases would also mean a better welfare.

Even if there is no official definition of animal welfare, there is a common ground for the definitions that were proposed by several groups of experts. For example, there is the concept of the "five freedoms" of the British Farm Animal Welfare Council, (today Farm Animal Welfare Committee, FAWC) (8). The concept was founded in 1979, and has since then been updated and revised several times. The five freedoms are as follows: "Freedom from hunger and thirst. Freedom from discomfort. Freedom from pain, injury or disease. Freedom from fear and distress. Freedom to express normal behaviors." Webster also applied the concept of the five freedoms to livestock (9).

A description of the term also used by the O.I.E (World Organization of Animal Health) and created by Broom is that "the welfare of an individual is its state as regards its attempts to cope with its environment" (10).

In order to be able to measure animal welfare, animal welfare indicators come into play. In general, they can be divided into resource-based, management-based and animal-based indicators. Resource and management-based indicators assess animal welfare through the animal's surrounding environment or housing and generally serve to prevent respective risks or threats. Animal-based indicators are results-oriented, evaluate animal welfare in the animal itself and thus provide a picture of the present status of the individual.

To evaluate welfare, tools are needed that can assess it in an objective, animal-based manner and are suitable for daily use. Many researchers have dedicated themselves to this task, so that there are now various evaluation systems, measurement protocols and other approaches.

One of the most popular assessment systems is the European Welfare Quality® Assessment Protocol, which contains an explanation of the procedure for evaluating the welfare of cattle (11). A working group of the German KTBL (Kuratorium für Technik und Bauwesen in der Landwirtschaft e.V.) also used the Welfare Quality® indicators to provide a guideline for the operational self-assessments (12).

In view of the large number of indicators, which are often difficult to measure, the question comes up, which indicators are most reliable and suitable for farmers daily self-assessment.

In this systematic review, the currently used indicators for the assessment of animal welfare in dairy cow farming are presented, discussed and assessed for their validity.

The focus is laid on animal-based indicators, because they can be successfully used in the evaluation of the welfare especially in the context of dairy cow farming in relation to laws, codes of practice, quality assurance schemes and management (13). Standardized valid animal-based welfare indicators could be able to improve the husbandry of dairy cows. The aim is also to provide farmers and other agricultural professions with assistance in evaluating animal welfare, as there is no clear definition at the legislative level.

2 Materials and methods

2.1 Databases and catalog of criteria

The literature research utilized five databases: CAB Direct, PubMed, and Scopus for English-language publications, and Google Scholar and Livivo for German-language literature. Publications in both German and English were considered. The publication years were restricted to the period from January 1, 2011 to October 20, 2021. In terms of content, the studies were limited to those geographically situated in Europe. Dairy cows

were identified as the sole relevant livestock group for inclusion in the systematic review.

2.2 Search terms

Due to variations in the operational and selection elements among the five databases employed, the search methodologies differed as follows: in CAB Direct and Scopus, descriptors were searched within the abstracts. In PubMed, descriptors were searched within both the titles and abstracts. In Google Scholar and Livivo, there were no restrictions; hence, the descriptors could appear anywhere within the full text. Additionally, citations and patents were excluded from the search in Google Scholar.

For the German-language searches on November 7, 2016 and October 20, 2021, the following terms and combinations were chosen in Livivo:

Tierwohl Milch*.
Tierwohl Rind*.
Tierwohl Kuh.
Wohlbefinden Milch*.
Wohlbefinden Rind*.
Wohlbefinden Kuh.
Tiergerecht* Milch*.
Tiergerecht* Rind*.
Tiergerecht* Kuh.

Note: Replacing the search term "cow" with "cows" returned identical results in Livivo, so the search was limited to the descriptor cow.

The German-language search on December 14, 2016 and November 5, 2021 in Google Scholar was carried out with the following terms and combinations:

Tierwohl.
+ MilchkuhORMilchküheORMilchrinderORKuhORKüheORRindORRinder.
- SchafORZiegeORKalbORKälberORGeflügelORHuhnORHühnerORPuteORSchwein.
Wohlbefinden.
+ MilchkuhORMilchküheORMilchrinderORKuhORKüheORRindORRinder.
- SchafORZiegeORKalbORKälberORGeflügelORHuhnORHühnerORPuteORSchwein.
- FerkelORMannORFrauORKindORMusikORReligion.
Tiergerecht.
+ MilchkuhORMilchküheORMilchrinderORKuhORKüheORRindORRinder.
- SchafORZiegeORKalbORKälberORGeflügelORHuhnORHühnerORPuteORSchwein.
Tiergerechtheit.
+ MilchkuhORMilchküheORMilchrinderORKuhORKüheORRindORRinder.
- SchafORZiegeORKalbORKälberORGeflügelORHuhnORHühnerORPuteORSchwein.

The English-language search in CAB Direct, PubMed and Scopus on August 23, 2016 was carried out with the following terms and combinations, with German publications also being permitted:

dairyORcow*ANDwelfareNOTgoatNOTsheep.
dairyORcow*ANDwell-beingNOTgoatNOTsheep.
dairyORcow*ANDwellbeingNOTgoatNOTsheep.

This search was repeated on October 7, 2021, in Pubmed, and on October, 21, in CAB direct.

2.3 Abstract-screening and grouping

In the initial phase, the results of the database searches were categorized based on publication type. Simultaneously, a software-assisted cleanup using Citavi Version 5 was conducted to remove duplicates from the result list. Initially, the database entries totaled 5,119, which were subsequently reduced to 3,491 after the removal of duplicates. Further manual sorting eliminated additional duplicates, resulting in a final count of 2,818 database entries.

Subsequently, these entries or publications were grouped and, where necessary, their bibliographic information was completed. Initial classification of the publications included the following groups: "wrong species," "wrong topic," "outside Europe," "uncertain relevance," and "potentially relevant".

Furthermore, a separate category labeled "completely irrelevant" was established, into which certain database entries were placed due to inaccuracies in the search algorithms and links to literature.

Additionally, entries pertaining to collective works and conference proceedings, which often serve as mere placeholders for individual titles, underwent cleanup. Some collective works required identification and linkage to existing individual titles, while others necessitated the creation of artificial entries to establish clear bibliographical associations. Ultimately, these placeholders were removed from the remaining publications, without altering the original number of hits retrieved from the database query.

All titles were systematically categorized based on the exclusion criteria. The title of each publication, along with its abstract when available, was thoroughly reviewed. In cases where essential information was missing, references were made to the full text.

The categorization process followed a hierarchical approach. For instance, if a publication described the wrong species (e.g., pig instead of dairy cow), the topic and study location became irrelevant. Incorrect life stages, such as calves, were also sorted out. Priority was given to species, followed by subject matter, and then study location.

Publications primarily addressing dairy cow owners or producers of dairy products and their perspectives on animal welfare were classified under the "wrong topic" category. Conversely, publications focusing on farmers' attitudes and assessments of animal welfare in general, without specific emphasis on dairy cows or covering other livestock species, were considered "wrong species".

Any database entries not related to animal welfare, well-being, emotions, or behavior specifically in dairy cows were excluded as "wrong topic".

Furthermore, all ambiguous and potentially relevant publications were classified into original studies (peer-reviewed), reviews, or knowledge transfer (book chapters, guidelines, and other forms of "gray literature"). The subsequent analysis focused on original studies within these two categories.

2.4 Verification

The assessment of the potentially relevant publications as ultimately relevant or not was carried out objectively by three scientists. 125

publications were verified and clearly assigned at this point. The verification process is illustrated in the form of a flowchart in Figure 1.

2.5 Risk of bias

The risk of bias was reduced to a minimum through the systematic approach and the creation of uniform search criteria for the literature search. In addition, the risk was reduced by the fact that 3 independent scientists evaluated using the same inclusion and exclusion criteria which were previously determined.

Quality assurance is ensured by the fact that all included studies underwent peer review and were additionally evaluated according to the "Standard Quality Assessment Criteria for Evaluating Primary Research Papers from a Variety of Fields" (14). For this purpose, the "Checklist for assessing the quality of quantitative studies" was utilized, and all papers were assessed based on the following criteria:

- Question/objective sufficiently described?
- Study design evident and appropriate?
- Method of subject/comparison group selection or source of information/input variables described and appropriate?
- Subject (and comparison group, if applicable) characteristics sufficiently described?
- Outcome and (if applicable) exposure measure(s) well defined and robust to measurement/misclassification bias? Means of assessment reported?
- Sample size appropriate?
- Analytic methods described/justified and appropriate?
- Some estimate of variance is reported for the main results?
- Controlled for confounding?
- Results reported in sufficient detail?
- Conclusions supported by the results?

Since all papers had already been confirmed as thematically suitable for the systematic review by the criteria mentioned beforehand, none of the papers were excluded, even if they received a low score. The quality assessment was not intended for further exclusion, but rather for evaluating the quality of the studies. A lower score can also be explained by a different format of the respective paper and does not necessarily mean that the quality of the paper is insufficient.

Scores of 2 were assigned for "Yes," 1 for "Partial," and 0 for "No." If nothing applied, "N/A" could be used for some of the criteria. The scoring was conducted by Author 1 and Author 3.

In the systematic review, papers numbered 15 to 139 in the list of citations were included. Among these, the highest attainable score is 22, which has been achieved by 4 papers. Notably, 73% of the papers scored 19 or higher, indicating a generally high level of quality across the included studies. Conversely, a small proportion, specifically 4.8% of the papers, scored 12 or lower. It's worth noting that the paper with the lowest score of 7 is categorized as a research reflection. This lower score may be attributed to the fact that the checklist questions may not be entirely suitable for evaluating this particular type of text. This also applies to one paper, which scored 11 (ranking 49 in the list of citations), and another paper which scored 12 (ranking 53 in the list of citations), as they are reviews. Additionally, Paper 44 in the citations list also scored 11. This could be attributed to the study's described inconclusive correlations, which can result in a lower score. The systematic review's inclusion

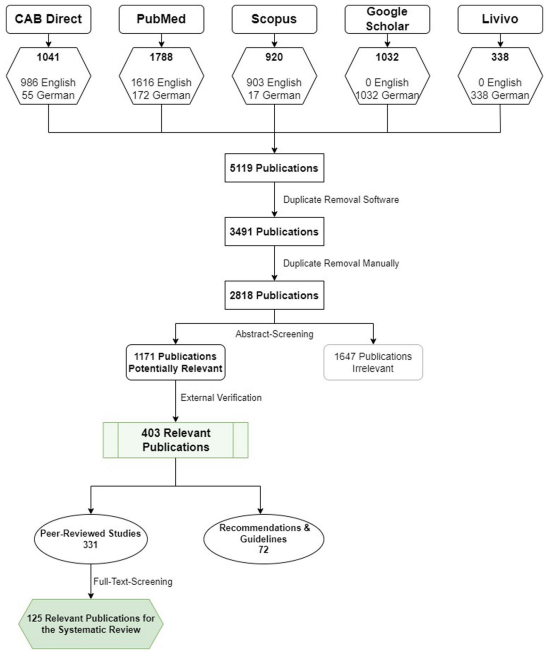

FIGURE 1
Selection process of research papers.

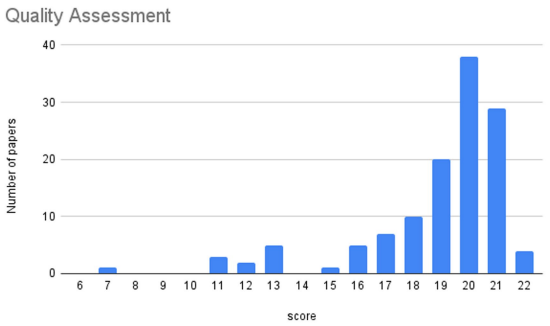

FIGURE 2
Quality assessment.

of a broad range of studies, with careful consideration of their quality and relevance, provides a comprehensive and reliable synthesis of the available research. This thorough approach ensures that the conclusions drawn from the review are well-founded and reflective of the overall evidence base. The distribution of the results of the quality assessment is shown in Figure 2.

3 Results

The systematic review yielded a comprehensive overview of studies across various topics related to welfare indicators. The frequency of each topic/category within the literature is quantified as shown in the following bar chart. The frequency of each topic/category within the literature is quantified as shown in Figure 3.

Out of the selected studies, a significant amount refers to the WQ® protocol, with 32 papers dedicated to this topic. The second most

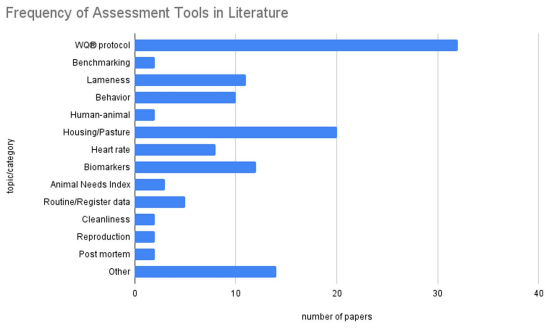

FIGURE 3
Frequency of assessment tools in literature.

common category is "Housing/Pasture," with 20 papers. The topic of biomarkers is also frequently addressed, appearing in 12 papers. "Lameness" with 11 papers and "Behavior" with 10 papers are also significant topics in the measurement of welfare. The category "Other" consists of papers that could not be assigned to any of the specific categories and for which there was only one paper, making it impossible to form additional categories. The "Animal Needs Index" was examined and applied in 3 papers. Less frequently discussed topics include "Human-Animal Relationship," "Benchmarking," "Cleanliness," "Reproduction," and "Post-mortem," each addressed in 2 papers. It is worth noting that "Cleanliness" is a component of the WQ® protocol and therefore is applied more frequently, though not as a standalone indicator, but in combination with other indicators. The following sections present the results of the research in detail.

3.1 Welfare quality® protocol

One of the most important assessment approaches in the EU is the Welfare Quality® protocol. Although there is no gold standard for evaluating animal welfare, the WQ® protocol is very often referred to. It consists of 30, mostly animal-based welfare indicators and is divided into four principles: good housing, good feeding, good health, and appropriate behavior (11).

The WQ® protocol is widely accepted and has been validated in several studies (15–19) and proven to be useful. Gieseke et al. (15) applied the WQ® protocol as part of a field study and statistically analyzed the data to evaluate the WQ® protocol. They could prove that the WQ® protocol offers good prerequisites for recognizing farm-specific risk factors and recording animal welfare at farm level (15).

The WQ® protocol has been successfully used in various studies (15–19) to measure animal welfare. For example, Coignard et al. (16) showed, that the overall health of dairy cows (130 farms were assessed) was moderate but ranged with the farming system. In a study from Macedonia in 2014 (17), it was disclosed that the most welfare concerns are ascertained in the WP Good Feeding and Good Housing. Another study, which investigated three dairy cow farms concerning the common health disorders, assessed the three farms as "acceptable," which means that the provided welfare circumstances performed the minimum needs of animals (18). Several health problems were assessed which differed between the farms. One farm had more occurrences of skin injuries than the other two. Other detected problems were for example reproductive disorders and lameness (18). In another paper, the assumption that "monitoring of welfare could increase the profitability of dairy herds by improving indices of reproduction" was tested and the authors found remarkably positive correlations between welfare parameters, reproductive indices and milk production (19). In 2020, Bugueiro et al. studied 31 dairy herds and used the WQ® protocol to identify fields in which the surveyed herds should improve (20).

In an approach (21) on 34 Austrian dairy farms, the farms were assessed two times within 1 year. The farmers received a written report and were invited to apply improvement measures in husbandry and management. The result was an implementation rate of 57% of the recommendations, a notable refinement of udder health and cleanliness of teats, but no improvements in leg health (21). One point in which many experts seem to agree, is that the execution of the WQ® protocol is very time consuming (22–26) and takes a full day (6-8 h) to perform. This circumstance makes the application of the WQ® protocol expensive (22, 24) and resulted in various attempts to change or shorten the WQ® protocol without changing the accuracy of the measurement.

The Danish Cattle Federation (DCF) developed a simple welfare assessment protocol and compared it to the WQ® protocol (22). The new DCF protocol consists of 14 measures, 13 of which are animal based, and that takes 2 hours to apply. In an extended version, it reached a significant correlation with the WQ® protocol on all levels. Despite the overall high correlation, some specific areas showed only moderate correlation. For instance, "water provision" in the extended DCF protocol showed moderate correlation due to differences in how fat animals are considered. Similarly, for "positive behavior," the DCF protocol uses avoidance distance as a measure, which does not fully capture the aspects included in the WQ® protocol, such as grooming and pasture access.

The extended DCF protocol's use of simpler, animal-based measures, and fewer cows inspected (16% compared to WQ®'s 38%), makes it more practical and time-efficient for routine farm use. However, this comes with the risk of false positives and negatives in welfare assessments. Despite this, the time saved and the practical focus on animal-based measures make the DCF protocol a viable alternative.

The DCF protocol uses relative percentiles based on the population, which will change as welfare levels change, unlike the absolute scores of the WQ® protocol. This makes the WQ® more suitable for cross-country comparisons and labeling, but the DCF's simpler summarization method is more transparent and user-friendly.

The extended DCF protocol was developed specifically for Danish conditions and worked well for cattle in Denmark (22).

Another approach to reduce the time for assessment was to identify the so-called "iceberg-indicator," which is believed to provide an overall assessment of welfare. The paper concludes that by only measuring one single, resource-based score, the absence of prolonged thirst (WQ® criteria score), the correct welfare classification can be obtained in 88% of the cases (23). The assessment time took 15 min.

A further attempt to reduce the assessment time was presented in a paper from van Eerdenburg et al.: to exchange the most time consuming parts of the WQ® protocol, like for example the behavioral observations, by environment-based measures and other modifications (25). It succeeded as a practical tool that takes 1,5 h to perform on a farm with 100 cows.

In the WQ® protocol, Qualitative Behavior Assessment (QBA) (11) is a method used to evaluate animal welfare based on observers' subjective scoring of behavioral expressions such as posture, activity level, and facial expressions. It provides a holistic understanding of animal welfare on farms by considering the overall impression of behavior rather than focusing on specific behaviors or physiological parameters (11).

In 2012, a paper was published in which the authors also tried to solve the time problem by using QBA as stand-alone assessment approach to determine farms with limited welfare conditions before performing the whole WQ® protocol (26). No significant correlations could be detected, so the study does not recommend the use of QBA as a single measurement tool.

The next approach is also a reduction of the WQ® protocol, in which four different assessment methods were used: avoidance distance at the feeding rack; QBA; behavioral observations and clinical observations. The conclusion was that it is not recommended to leave out indicators of the WQ® protocol, but to use additional data or automated monitoring systems in terms of time-reduction (24).

Another method to make the WQ® protocol more user-friendly was presented by Tuyttens et al. (27): they used only a few key measures to assess the welfare of the animals, and combined them into a single welfare index (WI). The indicators were determined through expert surveys (lameness, leanness, mortality, hairless patches, lesions/swellings, somatic cell count). The simplified protocol turned out to be consistent with the opinions of experts and the time needed to carry out the assessment was reduced by a factor of 2–3. The authors recommended to include a disclaimer that outlines both positive and negative effects that may not be accurately detected by the current set of measures (27).

Van Eerdenburg et al. (28) developed a scoring system for free stall barns to observe the dairy cow comfort and examined the impact on the milk yield. They took animal-based parameters and environmental aspects and found that they needed significantly less time to apply the system compared to the WQ® protocol. A positive correlation between the used free stall parameters and milk yield was found (28).

Studies using databases and epidemiological approaches have explored various aspects of dairy cow welfare. Otten et al. (29) aimed to construct animal welfare indices (AWI) from data of 73 Danish dairy herds to compare and validate register- and resource data against animal-based data, concluding that on-farm animal welfare assessments with animal-based indicators were more reliable due to limited correlations between indices and predictive key indicators (29). In a study from 2015 (30), a national database was used in order to find dairy cow farms with insufficient animal welfare conditions. Out of this database, which contains registrations of cows and their deaths and movements, 14 million records were evaluated to discover and figure out 15 possible welfare indicators. An on-farm welfare assessment with the WQ® protocol was carried out on 24 farms for comparison. In conclusion, the two variables "proportion of on-farm deaths" and "calving-to-calving-interval" helped to identify farms with poor welfare (30).

Accurate welfare measurement was discussed by assessing different sampling strategies, showing that low-prevalence measures required more cows for accurate estimates (31). In a 2014 study (32), an epidemiological approach was used to investigate welfare issues in French dairy cows, identifying pain, bad health, and poor resting comfort as significant problems (32).

Kirchner et al. assessed 30 dairy farms (33) using the WQ® protocol, identifying weaknesses like injuries and discomfort in lying areas, and noting that organic and low-input systems can achieve good welfare results, though access to pasture did not always meet "Excellent" standards (33).

In their study, Wagner and colleagues (34) found that organic farms scored higher in all WQ® principles compared to conventional farms, but both showed room for improvement in "Good Health" (34).

A 2020 study examined the influence of cubicle traits on animal welfare, finding bedding type to be the most influential factor (35). Popescu et al. compared tie-stall and free-housing systems, finding that most evaluated farms had "unacceptable" welfare, with insufficient water supply being a major issue (36). The impact of the daily grazing time of cows on their welfare was determined (37) and the results showed that in farms where grazing was available, the welfare of cows improved from winter to summer. Positive effects cannot be ensured if the general management fails to meet the requirements of the cows (37).

In 2015, the replicability of QBA outcomes was examined, across three distinct observation periods throughout the day (early morning, late morning, early afternoon) (38). For certain farms, QBA results may differ considerably based on the time of day when the evaluation is conducted. As recommended in the WQ® protocol, using a standardized observation time facilitates observing the animals under similar conditions, thereby ensuring a high level of comparability (38).

In the following study, the reliability of QBA for assessing the welfare of dairy cattle was examined by analyzing videos and comparing the observations of experienced and inexperienced observers (39). The results showed that the agreement between different observers varied from slight too high for individual QBA descriptors and from slight to moderate for QBA scores. Additionally, there were differences in the values assigned by experienced and inexperienced observers for half of the descriptors and the QBA score (39).

In the following study, the authors asked whether people who are trained in using the WQ® protocol for dairy cattle have the same opinions as the scores calculated by the WQ® protocol (40). Their findings revealed that certain measures that were deemed less important by experts had a greater impact on the overall welfare categorization of the WQ® protocol. Conversely, measures that were considered highly important by experts had a lower effect on the overall welfare categorization. Specifically, measures related to drinkers had a significant impact on the welfare categorization, while these related to lameness and mortality had a lower effect (40).

In a paper from 2020 (41), it was also considered whether the WQ® protocol could be implemented with sensor technologies. It was stated how current precision livestock farming technologies have the capability to evaluate the majority of WQ® indicators. Although certain welfare indicators may not be suitable for sensoring technologies, alternative measures that evaluate the same welfare criteria could be used as a substitute. It is expected that in the future, there will be an increase in the availability of objective and continuous data provided by precision livestock farming technologies (41).

Four scientists analyzed three widely recognized systems (WQ®, FARM and The Code of Welfare), highlighting their strengths and weaknesses (42). Expanding the scope of environmental measurements could potentially enhance the ability of WQ® to identify the environmental factors that impact the welfare outcomes observed in cows (42). De Vries et al. focused on the key welfare

measures influencing WQ® classification in Dutch dairy herds, suggesting a need to reconsider the role of expert opinion and the algorithmic operator to improve classification (43).

A survey on 131 French dairy farms using the WQ® protocol identified major welfare issues, emphasizing the importance of farm-specific characteristics in welfare plans and continuous improvement in health, behavior, and feeding (32).

As an innovative approach, hair cortisol concentration, reflecting long-term stress, has emerged as a potential indicator (44). However, the correlation between Welfare Quality® scores and pooled hair cortisol concentrations remains inconclusive, necessitating further research with larger sample sizes and standardized protocols, according to a study by Vesel et al. Various factors, including sampling time, cow characteristics, and environmental conditions, influence hair cortisol levels, demanding meticulous attention in future studies (44).

Bergschmidt et al. (45) had the idea of including an approach in the EU's Rural Development Program, where the payment for the farmers is depending on the results. So far, the payments have been based on actions like a welfare friendly housing or management, neglecting welfare outcomes. In a multi-step process, which involved a literature review, a written Delphi survey with scientists, a group discussion with stakeholders („practioner workshop") and on-farm trial, 10 indicators were selected. An assessment of the WQ® protocol has also taken place. The project indicators and the WQ® protocol exhibited a limited level of coherence in their comparison.

It is said that dairy cattle support measures can cover all aspects of animal welfare, including health, behavior and emotions, with the help of action-oriented requirements and outcome-oriented indicators in combination (45).

In 2017, a paper was published with the aim to demonstrate the necessity of adjusting some elements of the WQ® protocol in tropical regions, so it would be even more useful (46).

3.2 Benchmarking

Benchmarking plays a role in evaluating and enhancing the performance of dairy cow farms by comparing their practices and outcomes to industry standards and best practices. In 2018, a welfare assessment protocol has been created specifically for small-scale dairy cattle farms that practice vertical transhumance (47). It is based on the WQ® protocol. In a sample of 67 farms, 18 nonbehavioral animal-based measures were evaluated before, during, and after the mountain pasture period. The purpose of this study was to present field data from the transhumant system and to identify intolerable welfare affairs. To contribute to the discourse on achievable welfare results for the two husbandry conditions that define a transhumant system, a benchmarking exercise was conducted. The aim was to determine the comparative limits (thresholds for the lowest quartile) for each animal-based measure. The results show that a significant number of cows (65%) had bald spots before being put out to pasture. When cattle were housed indoors, there was a notable prevalence of 80% of them being found to be dirty. Additionally, more than 13% of the cows were identified as very thin (47).

Trillo et al. successfully used a benchmarking process in 73 dairy farms in Spain to detect negative points and improvable aspects. Animal-based indicators were assessed, which led to the outcome that hock lesions and lameness are common problems, also like a suboptimal Body Condition Score (BCS) (48).

3.3 Lameness

A review from 2011 emphasizes the behavioral implications of lameness, exploring the interplay among locomotion scores, lying patterns, and milking parlor positioning (49). The presence of clinical lameness not only induces chronic stress but also has an effect on reproductive hormones and sexual behavior. Hoof diseases, contributing to pain, further jeopardize overall welfare. Enhancing comfort, especially in lying areas, emerges as a key strategy to mitigate lameness and promote holistic health, with straw bedding demonstrating notable advantages. Nonetheless, it's crucial to recognize that relying solely on measuring lying behavior may not provide an accurate gage of lameness severity (49).

O'Connor et al. defined the quality of mobility by investigating the connections among particular mobility scores, claw disorders, BCS and cow parity (50). Data was gathered for 6,927 cows from 52 dairy herds. These data encompassed mobility scores („0 = optimal mobility; 1, 2, or 3 = increasing severities of suboptimal mobility "), the type of claw disorders, the BCS, and the parity of each cow. Based on the results, it's apparent that a correlation exists between mobility scores and claw disorders among dairy cows in pasture-based systems. Moreover, the research establishes links between BCS, cow parity, and mobility scores. Notably, claw disorders with severity scores <2 were tied to an elevated risk of developing mobility score 3 in contrast to score 0. This emphasizes the effectiveness of mobility scoring in identifying cows with mild claw disorders at an earlier stage (50). In another study, they correlated mobility scores with reproductive performance and production measurements (51). Their research indicates that poor mobility in dairy cows during spring-calving in pasture-based systems is linked to reduced production (lower milk, fat, and protein yields, along with higher somatic cell count (SCC)) and compromised reproductive performance (longer calving intervals) (51).

To further investigate "lameness," it is discussed how it impacts cow welfare through a comparison of regular and irregular gaits, including the utilization of Locomotion Scoring (LS) systems for detecting lameness (52). Implementing LS to identify lame cows demands clear gait feature criteria to enhance result consistency. However, practical use is constrained by the need for proper farm facilities to guarantee precise outcomes (52).

A review from 2012 analyzes how lameness affects the behavior of intensively managed dairy cows (53). Lameness influences social rank, with affected cows losing positions in the food trail and milking order. This impacts productivity and survival (53).

Concerning to Weigele et al., mildly lame cows show distinct behavior changes from nonlame ones, affecting lying, activity and feeding patterns (54). These alterations, like reduced movement and extended lying, impact physical well-being and energy balance, potentially leading to more health issues and shorter lifespans. Limited mobility may also weaken resilience and social behaviors. This underscores lameness' early and significant impact on animal welfare for moderately lame dairy cows in open housing (54).

Another study also explored the connection between lameness and changes in feeding behavior (55). Using gait scoring and monitoring feeding behavior, intake, milk yield, and weight, the

researchers found that cows with more severe lameness spent less time feeding daily. They used electronic feeding troughs and automatic milking systems (AMS) for the measurements. They also found an interaction between lameness score and parity, with severely lame first-calving cows feeding the least. Profoundly lame cows ate faster but had lower body weights in comparison (55).

Another approach was also carried out using AMS. These systems use technology to automate milking procedures, offering advantages in terms of efficiency, cow welfare and data collection.

Three studies from 2013 showed that lameness in high-yield cows within an AMS affects feeding, rumination, and AMS visits (56). This has negative implications for farm profitability and cow welfare. Further research is necessary to optimize AMS technologies for health monitoring (56).

In recent years, the utilization of technology in the dairy industry has extended beyond milking processes to encompass the area of cow welfare. Automatic lameness detection systems have emerged as a helpful tool. The aim of the next study was to develop and validate a model for detecting lameness based on daily activity data (57). Automated lameness detection can rely on daily fluctuations in animal behavior. Activity sensors that monitor parameters like lying time and bouts were employed to record behavior of every cow per day. The lameness detection model showed consistent results between development and validation sets. Sensitivity reached 85.5%, making the model practical, though 88.8% specificity might need enhancement. According to the authors of the study, behavioral shifts as indicators of lameness hold potential (57).

Nechanitzky et al. (58) also assessed indicators for automated lameness detection in cubicle barns. They involved 32 lame cows with one hind limb claw horn lesion and 44 healthy nonlame cows. Nighttime lying and standing behavior were recorded by accelerometers, hind limb weight distribution by weighing platforms, feeding behavior by nose band sensors, and heart activity by Polar devices (58). Locomotion score correlated positively with lying time and weight difference, negatively with limb weight ratio and deviation. The best predictor of lameness included weight deviation and lying time. They concluded that weighing platform data, with or without lying time, are valuable for identifying claw horn lesions in one hind limb lameness. Feeding behavior and HRV variables have minor relevance (58).

In a review by Leliveld et al. (59), the need to integrate various welfare indicators to create a comprehensive assessment of dairy cow welfare on farms is highlighted. The focus is on developing an integrated automatic system to detect issues like lameness, heat stress, and pain. The study identifies common indicators, such as reduced feed intake, suitable for detecting overall reduced welfare, and specialist indicators, like increased respiratory rate for heat stress. Combining these indicators offers the potential for an early warning system in addressing welfare problems (59).

3.4 Behavior

Dairy cow behavior serves as a window into the dynamics between animals and their environment within modern farming systems. By studying how cows interact, move, and respond to various stimuli, valuable insights into their well-being, health, and overall performance can be gained. In the context of three open cowsheds, a study from 2012 investigated the impact of the potentially stressful waiting area of a milking parlor on dairy cows' behavior and welfare (60). The research encompassed 3,522 individual cow observations. Waiting times, varying based on factors like group size and milking parlor capacity, reached up to 1 h, 42 min, and 22 s. Cowsheds I and II saw only around one-third of cows ruminating in the waiting area, while Cowshed III, with the smallest feeding group, shortest waiting time, and most space per cow, observed up to 52% of cows ruminating. Extended waiting times curtailed normal behavior opportunities for cows, indicating compromised welfare (60).

The study from Hedlund and Løvlie showed that links between personality traits and production are behavior-specific, influenced by milk measurements and breed (61). A common trend indicated that behaviors associated with cow nervousness were linked to reduced milk production. This alignment with resource allocation theory suggests negative correlations (61).

Two studies investigated the impact of omitting scheduled milking on cow comfort indicators (62). Decreased lying time, increased mammary pressure, and higher milk leakage resulted from reducing milking frequency from twice to once daily, either temporarily during lactation or weekly. Rapid behavioral and physiological adaptation, restoring parameters to pre-omission levels, were observed in both studies. Hence, immediate cow comfort wasn't significantly affected by transitioning to once-daily milking or skipping a weekly session. It is said that more research is needed to assess long-term effects on cow welfare (62).

There are several papers in which automated devices were used to collect data about dairy cow behavior. A study from Italy compared behavioral indices from diverse scan-sampling frequencies, focusing on lying and standing behaviors (63).Video recording of 69 cows' behaviors over a week, with Temperature Humidity Index (THI) logged every 15 min, unveiled insights. Results from hourly interpretations of lying, standing, and feeding behaviors, especially between daily milkings and evening hours, showed strong correlations to 10, 20, and 30-min scans. Night hours had limited impact. Farm management was significantly linked to cows' activity 1–2 h post-milking. Video systems proved effective for cow activity analysis (63).

Validating the AfiTagII device's accuracy in measuring lying behavior was the goal of another study (64). The device, attached to cows' hind legs, showed high correlation with direct observations of lying time. Frequency of lying bouts had a positive predictive value of 0.96 for lactating cows on slatted floors and 0.85 for dry cows on deep bedding, compared to direct observations. The AfiTagII accurately estimates lying behavior in Danish Holstein and Jersey cows, regardless of bedding material or breed. However, skin lesions developed in some monitored cows, highlighting the need for device improvements (64).

Another approach used accelerometers to classify cow behaviors (65). Combining neck and leg data achieved precise (80–99%) and sensitive (87–99%) behavior classification. Neck accelerometers performed better for feeding (92% precision, 97% sensitivity) than leg ones (80% precision, 88% sensitivity). Classification accuracy depends on sensor position, sampling rates, and axes (65).

Using automated sensors, Ramón-Moragues et al. (66) tracked behaviors of 40 cows under varying heat stress conditions. The aim was to identify heat stress-induced behavior changes. All behaviors were affected by environmental conditions, and the cows adapted by modifying their actions. The sensors proved valuable in capturing

these adaptations, potentially paving the way for an early warning system based on behavioral shifts. Heat stress influenced behaviors like breathing, eating, resting, and activity. As the Temperature-Humidity Index increased, feeding, rumination, and resting times decreased, while panting and activity increased. Behavior patterns also changed during cooler times of the day (66).

Having explored technical devices to examine cow's behavior, the focus shifts to the significance of grooming substrates in promoting welfare. Providing grooming materials is said to address the natural behaviors of cows and shall contribute to stress reduction. McConnachie et al. (67) studied dairy cows' motivation for an automated mechanical brush. Cows were taught to unlatch a weighted barrier for access to fresh feed (positive control), a mechanical brush, or an unoccupied area (negative control). They gaged the weight cows would push for each resource. Cows demonstrated comparable motivation for fresh feed and the brush, despite varying data collection approaches, with lower motivation observed for the empty space (67).

In their invited review, Tucker et al. (68) delve into the factors that influence cows' motivation to lie down and explore the consequences for their health and overall biological function when this behavior is impeded. The research sheds light on a range of environmental and cow-based factors that impact lying time, underscoring the significance of offering appropriate lying areas on farms to enhance animal welfare. Although increased lying times typically signify cow comfort, exceptions may arise due to factors such as disease or specific behaviors. When evaluating animal welfare based on lying time measures, careful consideration of individual contexts is essential (68).

In a thematically related study, Vanhoudt et al. (69) aimed to assess the variability of the indices "cow rumination" and "lying behavior" in a herd with an automatic milking system under stable husbandry conditions. Over 28 days, standing index, cud chewing index, and rumination index were monitored. The lowest variation occurred between 240 and 270 min after cubicle bedding refreshment for standing and rumination indices, and between 120 and 150 min for the cud chewing index. Despite consistent practices, there was still significant variation, suggesting the need for repeated measurements over consecutive days for reliability (69).

3.5 Human-animal relationship related indicators

Exploring the human-animal relationship within the dairy cow industry is important and has influence on choices for animal welfare and ethical considerations.

In alpine traditional husbandry systems, Battini et al. (70) examined the durability of Avoidance Distance (AD) tests as a means to evaluate dairy cow-human interactions over the long term. However, in this study, the consistency of AD varied throughout the year due to the distinctive nature of these traditional alpine systems. After the grazing period, the avoidance distance tends to be higher. This is attributed to significant shifts in the quality and quantity of human-animal relationship (70). Haskell et al. (71) posed the question: "Is the response to humans consistent over productive life in dairy cows?". Unpleasant interactions can affect welfare and productivity, prompting the inclusion of fear-of-humans tests in welfare assessments. However, practicality limits testing all animals on large farms. For sub-sampling, age impacts responses, shown by testing 114 Holstein cows across various productive stages. Cows became more approachable and less nervous with age until mid-1st lactation. Consistent rankings within groups across stages were observed (71).

3.6 Housing/pasture

The choice between housing and pasture systems for dairy cows is an important decision in modern agricultural practices. Striking the right balance between confined housing and access to open pasture directly influences animal welfare and milk production. There are a lot of different approaches to this topic.

A review published in 2016 compared the welfare of dairy cows in continuous housing and pasture-based systems (72). Despite advocating for continuous housing, pasture-based systems generally offer better welfare and health. Pasture-based cows have less lameness, hoof issues, lesions, and diseases compared to continuous housing of cows. Pasture access improves behavior, lying/resting times, and reduces aggression. Cows prefer pasture over indoor housing, especially at night. Yet, challenges include a negative energy balance and weather exposure in pasture systems. In conclusion, incorporating pasture access brings significant animal welfare benefits to dairy production (72).

The authors of the next paper also wanted to find out which form of husbandry would be better: pasture-based vs. confinement-based management (73). They used a three-sphere framework – biological functioning, natural behavior, and affective states – to assess wellbeing. Pasture-based cows have lower risks of various health issues, including mastitis, claw lesions, and lameness, but higher risks of internal parasitism and malnutrition (73). They also exhibit more normal behavior patterns. However, pasture-based cows might face challenges such as extended periods away from pasture and climate-related stress. Hybrid systems can alleviate negative effects by combining confinement and pasture elements. Ultimately, an optimal system allows cows some choice between environments, with effective management being key to ensuring good welfare (73).

A study from 2012 aimed to assess the impact of summer grazing on the welfare of dairy cows in contemporary cubicle loose-housing systems (74). The within-herd comparison of 41 Danish dairy herds revealed that summer grazing significantly improved overall cow welfare compared to full-time winter housing. The welfare index (WI) was lower in summer, indicating better welfare, with improvements in integument condition, claw conformation, and better access to water and food. The study suggested that many daily grazing hours were more beneficial than fewer hours for dairy herd welfare, emphasizing the positive effects of summer grazing on cow well-being (74).

In temperate regions, where cows graze on pastures, limited access to grass could lead to nutritional deficits, possibly affecting their wellbeing (75). A study from 2015 examined how daily herbage allowance (DHA) affects dairy cow behavior, locomotion, and hoof health. Cows were assigned to eight treatments based on experimental duration (2 or 6 weeks) and DHA levels (60, 80, 100%, or 120% of intake capacity). While daily lying time remained consistent, DHA influenced the duration of lying bouts, with higher DHA linked to shorter bouts. No significant effects were found on locomotion or hoof health. Although altered behavior and locomotion may not directly imply impaired welfare, they could indicate hunger or potential hoof issues. This research offers valuable insights for further exploration

into hunger-satiety status and hoof health, aiding in improved dairy cow management in intensified pasture-based systems (75).

Exploring welfare markers in dairy cows across distinct loose housing arrangements (deep litter vs. cubicle barns) using recycled manure solids as bedding material, the research of Molina et al. (76) uncovered vulnerabilities in feeding and health indicators within both housing types. The comprehensive welfare evaluation, considering feeding, shelter, and health metrics, revealed no distinguishable variations between farms implementing deep litter or cubicle barns. This implies the potential to attain favorable welfare circumstances regardless of the selected housing (76).

In the following examination, the welfare of dairy cows in Ireland's spring-calving, pasture-based systems during grazing and housing periods was explored (77). Seven welfare indicators were analyzed on 82 farms. Lameness, BCS, and tail injuries were issues, but ocular health was positive. Nasal discharge was lower during housing. Cows showed avoidance behavior in response to humans. Opportunities for improvement were identified, and top farms set benchmarks: 0 to 5% clinical lameness, 0 to 12% cows with BCS outside range, 0 to 27% ocular discharge, 2 to 16% nasal discharge, 0% tail injuries, 0 to 14% integument alterations, and 4 to 74% avoidance distance of >1 m. These targets can enhance cow welfare in spring-calving pasture-based systems (77).

In another approach, lying and walking activity of 29 cows was monitored using pedometers (78). Over 18 days, observations were conducted during pasture access and indoor housing periods. Pasture-grazing cows exhibited lengthier lying periods with fewer bouts, suggesting enhanced comfort and reduced restlessness. Outdoors, lying behavior was more synchronized, with the majority of the herd lying down simultaneously (78).

Heinz et al. (79) aimed to explore the relationship between claw health in dairy herds and various herd parameters, focusing on housing conditions. Data from four large dairy farms in northeast Germany, covering 18,119 observations of 3,690 cows, indicated that effective herd health management significantly improved claw health. The analysis revealed that farms with solid concrete flooring and deep-bedded cubicles had lower risks of claw disorders compared to those with concrete slatted floors and high cubicles. The frequency of functional hoof trimming, carried out two or three times per year, positively influenced claw health. The study emphasized the importance of optimal housing conditions and meticulous herd management in reducing the risk of claw lesions in dairy cows (79).

Twenty-nine Holstein-Friesian dairy cows experienced 18 days of overnight pasture access and 18 days of continuous indoor housing in a crossover experiment (80). Cattle learned to move towards a bucket location that offered a reward, while avoiding an unrewarded one. They were then presented with intermediate "probe" buckets. Probing these buckets indicated optimism in judgment, reflecting positive emotions. Although probe bucket approach latency did not differ between treatments, cows took longer to approach the known rewarded bucket with pasture access than indoor housing. These results suggest that pasture access in cattle reduces anticipation of known rewards compared to indoor housing, potentially leading to more positive emotional states in pasture environments (80).

Popescu et al. (81) compared the welfare of dairy cows in loose housing vs. tie-stall systems and test the hypothesis that loose housing leads to better welfare. Altogether, 2,624 milking cows on 60 commercial farms were evaluated using measures from the WQ® protocol. Notable differences were observed in most parameters and welfare principles, favoring the loose system. Tie-stall farms were mainly acceptable, while most loose housing farms were categorized as enhanced (81).

Another study compared the welfare of dairy cattle in different housing systems across six farms (82). Results indicated that the loose housing system had advantages in terms of cow comfort and health. The tie housing system showed higher indicator values of discomfort and management gaps related to hygiene and disease (82).

By comparing welfare between two tie-stall housing systems: those with and without outdoor exercise, significant differences were observed, indicating exercise positively impacts tethered cows' welfare. Farms allowing outdoor access had better welfare scores than those with permanent tethering, except for hunger and social behaviors (83).

In a paper from 2014, the authors assessed human-animal relationships (HAR) in dairy farms with tie stalls and loose housing (84). Observations and tests on 424 cows showed that tethered cows tend to be calmer, trusting, and less fearful of humans compared to loose-housed cows (84).

The influence of different bedding materials on well-being was examined in a study from 2014 (85). In farms utilizing straw bedding, dairy cows exhibited cleaner flanks, upper hind legs, tails, and udders compared to those with sawdust bedding. A greater proportion of cows in straw bedded farms had hairless patches on their tarsus area than in sawdust-bedded farms. The assessment of overall cow welfare across the visited farms resulted in either enhanced or acceptable ratings. More farms using sawdust were classified as enhanced, while those using straw were categorized as acceptable for cow welfare (85).

De Vries et al. (86) wanted to discover and compare the effects of housing and management factors on the occurrence of lameness, lesions or swellings, dirty hindquarters, and displacements in dairy cows housed in free-stall systems. The research identified 15 significant factors related to these indicators of cattle welfare. Notably, the condition of the lying area and access to pasture were linked to the prevalence of lameness, lesions or swellings, and dirty hindquarters. While no common factors were found for displacements and lameness, lesions/swellings, and dirty hindquarters, these indicators were primarily influenced by the quality of walking and lying surfaces. The frequency of displacements was associated with factors linked to limited resources (86).

By utilizing measures like "Body condition score" and "Cleanliness of observed body parts," the evaluation of dairy cows' well-being in permanent tie-stalls versus those with pasture access has effectively emphasized the significance of high-quality housing (87). This, in turn, enhances animal performance, impacting their health and productivity positively. The assessment of QBA has underscored the value of granting animals freedom and the opportunity for unrestricted movement, enabling the natural display of physiological behaviors (87).

A pilot study from 2017 aimed to compare the welfare of dairy cows in tie-stall (TS) and open-stall (OS) systems (88). Various health and stress-related parameters were measured in 80 lactating cows across eight farms. The study found that the housing system influenced certain indicators like ALT (alanine- aminotransferase), BHBA (β-hydroxybutyrate), OFR (oxygen free radicals), and cortisol levels, with OS showing higher OFR potentially due to increased productivity demands. Overall, while some parameters were affected, no significant signs of suffering were observed in either system, leading to the

conclusion that the tie-stall system did not display notable welfare issues when compared to open-stall (88). In another study on the same topic, metabolic, immunological, and stress-related parameters in 155 cows across 18 farms in Tuscany were analyzed (89). Results revealed that the housing system influenced several parameters, with oxygen free radicals (OFR) levels higher in the OS system, likely due to increased productivity. Cortisol levels did not suggest chronic stress. The study concluded that, based on physiological parameters, cows in the TS system showed no severe signs of impairment. Notably, parameters like lysozyme (SL) and OFR had more favorable values in the TS group compared to OS, and no evident distress signs were observed in either group (89).

Two traditional farming systems (semi-intensive and intensive) in Sicily were also examined (90). Using a multicriteria system based on the European Food Safety Authority (EFSA) model, 18 dairy farms were assessed for welfare and health measures. Overall, the study concluded that the semi-intensive approach in Sicily better meets animal welfare conditions compared to the intensive system (90).

Improperly designed cubicles can lead to skin problems, lameness, and dirtiness (91). While recommendations from the International Commission of Agricultural and Biosystems Engineering exist, their effectiveness varies. The paper of Lardy et al. aims to enhance these recommendations by analyzing cubicle features and their relation to cow welfare indicators across 76 farms with 2,404 cows. The results highlight key factors such as obstacle placement, bedding material, and cubicle dimensions that impact cow welfare (91).

3.7 Chewing muscle activity

The sensor system designed to quantitatively and qualitatively assess chewing muscle activity in dairy cows, is called DairyCheck (92). It employs Electromyography (EMG) principles, with skin-affixed electrodes gaging potential shifts during chewing muscle contractions (masseter). This facilitates personalized quantitative and qualitative evaluations, forming a basis for early ailment detection. Results demonstrate minimal variations in individual chewing phases. Daily chewing spans around 7 h, comprising roughly 15 phases each lasting about 28 min. Notably, nocturnal chewing variances are less pronounced, potentially aiding the detection of significant behavioral shifts during the night. The DairyCheck demonstration underscores its capacity to distinguish chewing from other oral actions. Further exploration aims to characterize eating-related oral activities and distinguish "other activities," with the goal of deducing feeding behavior from muscle activity (92).

3.8 Heart rate

As sentient beings, dairy cows experience a range of emotions and physical responses to their environment and overall health. Monitoring the heart rate of dairy cows as a tool for dairy farmers and researchers to assess their welfare has been the subject of numerous studies. This non-invasive and real-time measurement offers insights into various aspects of a cow's life, including its response to stress, pain, and environmental conditions.

Data was collected from 219 Holstein cows in different types of farms to study the impact of posture, rumination, and feeding on heart rate (HR) and heart rate variability (HRV) (93). The study found that sympathetic activity increased in the following order: when cows were lying, ruminating while lying, standing, ruminating while standing, and feeding. The vagal activity decreased in the same order in both smaller and larger-scale farms. The study also found that cows in larger-scale farms had lower vagal activity but higher sympathetic activity compared to cows in smaller-scale farms, suggesting potential welfare concerns related to social stress (93).

Erdmann et al. (94) aimed to investigate whether HRV parameters could serve as early indicators of metabolic stress in high-performing dairy cows. The researchers focused on evaluating the autonomic regulation and stress levels of 10 pregnant dried-off German Holstein cows throughout a 10-h fasting period, examining their conditions before, during, and after. They found that by examining HRV frequency domain parameters, cows could be retrospectively grouped based on their response to food removal, with some showing increased parasympathetic activity and others showing decreased activity. These findings suggest that HRV parameters could potentially be used as predictive markers for detecting alterations in autonomic regulation before metabolic disturbances occur (94).

A study was conducted on dairy cows milked in a high-capacity rotary milking system to assess their stress responses during the milking process (95). The researchers analyzed HR, HRV, rumination behavior, and step behavior during different stages of milking. The findings indicated that driving the cows to the holding pen caused an increase in HR and a decrease in vagal tone, while being in the holding pen resulted in decreased vagal tone and increased sympathetic tone. However, during milking, there was a recovery of autonomic activity, with increased vagal tone and decreased sympathetic tone, along with a low frequency of steps and a high prevalence of rumination, suggesting potential welfare benefits of the rotary milking system (95).

According to Hunter et al. (96), analyzing dairy cow sleep patterns is crucial for understanding their well-being amid environmental changes or other stressors. The current gold standard, polysomnography (PSG), can be challenging to conduct. In the study from 2021, HR and HRV were compared with PSG in two dairy cow groups, considering the impact of lying postures. Results showed HR decreasing with sleep depth, higher HRV during REM sleep, and lying postures influencing HR and HRV. Patterns were consistent across both groups, suggesting that HR and HRV changes correspond with sleep stages in cows. The findings also indicate associations between sleep stage, HR, and HRV, emphasizing their practical use in identifying sleep stages in dairy cows and enhancing accessibility for animal welfare research (96).

In another approach, sleep stages were monitored in 19 Swedish dairy cows during different lactation stages (97). Using electrophysiological recordings, REM and non-REM sleep, drowsing, awake, and rumination were examined. Results showed variations in REM sleep during lactation, with the shortest duration observed 2 weeks post-calving. Significant differences in REM sleep bouts were noted between various lactation stages. Nighttime predominantly hosted REM sleep and rumination. The study emphasizes the importance of considering lactation stage in future dairy cow sleep research (97).

Jurkovich et al. (98) compared HRV in dairy cows in a small-scale dairy farm in Hungary during traditional parlor milking and later automated milking. The purpose was to assess stress related to milking type and human interaction frequency. Parlor milking involved more

frequent human contact and animal movement. The study found that automated milking appeared less stressful for cows, attributed to shorter post-milking restraint and reduced human interaction. The parameters measured included HRV, fecal glucocorticoid concentrations, and avoidance distance. The results suggest that automated milking may be less stressful for dairy cows, with potential implications for improving animal welfare in conventional milking systems (98).

In a literature review by Kovacs et al. (99), it is described that there are different studies that highlight HRV as a more precise indicator of autonomic nervous system activity in dairy cattle. Effective in detecting stress related to routine practices, pain, and milking, HR and HRV play a crucial role in understanding dairy cow welfare. Future research opportunities include evaluating milking as a stress source and exploring the impact of chronic stressors, emphasizing the need for ongoing studies to enhance our understanding and improve overall animal welfare (99).

In a study from 2013, HR and HRV during milking in a parallel milking parlor were investigated (100). The results showed that there was no significant difference in animal welfare between the reference period and the different phases of milking. However, HRV parameters were significantly affected by factors such as parity, breeding bull, and milk production. Primiparous cows were found to be more susceptible to the milking process compared to multiparous cows. Overall, the study suggests that the conventional milking process is not highly stressful for cows, but certain factors can influence their physiological response (100).

3.9 The role of biomarkers, milk parameters and cortisol in welfare assessments

According to a 2015 review, enhancements in animal productivity have compromised fitness, leading to increased susceptibility to diseases and reproductive issues (101). Future breeding strategies should aim to strike a balance between high production and health, relying on both validated and new biomarkers for insights into physiological aspects. These biomarkers play a crucial role in comprehending adaptation to diverse environments, thereby contributing to welfare assessment and refining management and breeding practices. Subsequent studies should focus on identifying welfare biomarkers, developing cost-effective monitoring techniques, and exploring variations among bovine dairy breeds. Automated technologies hold the promise of precise quantification of animal responses, while biomarkers of robustness guide breeding for resilient animals (101).

In another review titled 'Engineering to Support Wellbeing,' it is noted that current EU livestock policies prioritize the well-being of dairy animals, addressing challenges such as health issues and fertility conflicts (102). Despite technological advances, the productive lifespan of dairy cows is limited, emphasizing the complexity of their management. Assessing dairy animal welfare involves both objective and subjective measures. The presented DairyCare project aims to enhance well-being through technological advances, integrating biomarker-based, activity-based, and systems-level welfare technologies. The livestock sector's technological focus heavily relies on RFID devices for monitoring and managing cows. Precision Livestock Farming (PLF) integrates RFID, IoT (Internet of Things), and SNO (smart networked objects) to monitor animals for optimal production. PLF provides opportunities for enhancing animal well-being, with wearables like accelerometers and automated milking systems contributing to data-driven decision-making in livestock management (102).

A review by Zachut et al. (103) underscores recent endeavors in identifying fitness, stress, and welfare biomarkers in dairy cows, particularly markers linked to energy balance, oxidative stress, and production-related diseases. The paper also highlights the necessity for future research and technological advancements, specifically in integrating established biomarkers into automated systems for practical use by farmers and veterinarians. Collaborative efforts across diverse disciplines and the adoption of PLF are crucial for improving dairy animal performance, health, and welfare (103).

The following systematic review from 2021 aimed to review PLF technologies for real-time welfare assessment in dairy cattle (104). Out of 1,111 publications, only 42 studies on 30 tools met validation requirements. A market search identified 129 retailed technologies, but only 18 (14%) were externally validated. Accelerometers had the highest validation rate (30%), while cameras, load cells, milk sensors, and boluses had lower rates (7–10%). Validated traits included activity, feeding, physical condition, and health. Most tools were validated on adult cows, with non-active behaviors validated more frequently than active ones. According to the authors, PLF technologies currently have limited potential for assessing appropriate behavior in dairy cows, necessitating further validation studies, particularly in commercial herds, to enhance trust and applicability. Future research should focus on developing and validating PLF technologies for assessing appropriate behavior, as well as monitoring health and welfare in calves and heifers (104).

PLF technology was used to monitor variables like activity and vocalization in another approach (105). A study at a Dutch dairy farm aimed to correlate cattle vocalization with behavior, finding significant frequency differences during lying and ruminating. Adult dairy cattle had lower vocalization frequencies than heifers. Despite concerns about housing conditions affecting welfare, sound analysis showed potential as a dairy cattle management tool. The study recommended future research with better camera coverage and consideration of breed-specific vocalization variations (105).

In a comprehensive investigation, another study sought to unveil the reliability of milk yield as an insightful indicator of the welfare within dairy herds (106). Favorable connections emerged, linking milk production to reduced aggression among cows and a positive emotional atmosphere within the herd. The study encompassed 125 French dairy farms. However, a contrasting relationship was observed concerning good health, evidenced by instances of diseases and injuries. The interplay of these opposing factors yielded no conclusive correlation between milk production and the overall well-being of the herd. The research implies that, although adverse emotional experiences and suboptimal emotional states can adversely affect milk output, relying solely on milk yield is insufficient for gauging the comprehensive welfare of the herd, given its intricate interrelation with health issues (106). In a different approach, the experts came to the conclusion that collected bulk tank milk data might not be a reliable pre-screening tool for estimating dairy cattle welfare at the herd level due to very weak associations (107). Weak but statistically significant correlations were found between bulk tank milk parameters (somatic cell count, total bacteria count, urea, proteins) and welfare

scores. These correlations were influenced by factors like dilution of individual cow milk and inclusion of non-lactating animals in welfare assessments. Despite the known link between milk parameters and udder health, correlations with overall animal welfare scores were weak. Total bacteria count showed partial confirmation of a link between farmer practices and animal welfare. Urea content displayed weak positive correlations with welfare scores, while no significant associations were found between fat content and welfare scores (107).

Jerram et al. (108) investigated stress levels in dairy cows during a transition from conventional milking to an automatic milking system (AMS). Stress, measured through cortisol levels in saliva and hair, can impact immunity and reproduction. AMS, associated with higher milking frequency and yields, showed varying effects. Non-lame cows exhibited reduced salivary cortisol levels post-AMS, while lameness and pregnancy affected salivary, not hair, cortisol. Hair cortisol increased after installation, possibly seasonally. AMS improved production, udder health, and milk yield, with no overall increase in cow stress (108).

In a paper from 2020, the physiological stress levels in dairy cows on 25 German organic farms were investigated, assessing cortisol metabolite concentrations in feces (109). The results showed decreased cortisol metabolite levels on farms that did not separate diseased cows, possibly indicating reduced regrouping stress. Lower levels were also observed on farms with straw yards and generous lying space. Increased human-animal contact was associated with decreased cortisol metabolite levels. However, unexpected results, such as higher levels on farms that fed concentrates by hand, suggest the complex and multifaceted nature of stress physiology in on-farm conditions. Overall, the study highlighted the importance of factors like resting comfort, human-animal contact, and feeding practices in influencing physiological stress levels in dairy cows (109).

To further investigate the correlation between cortisol concentrations in blood serum (KoB) and other non-invasive measures like saliva (KoS), tears (KoT), milk (KoM), and feces (KoK) in cows, Heinrich et al. (110) subjected cows to sham foot trimming (sKB) as an acute stress model. KoB and KoT increased during sKB, reaching a maximum at 60 min, while KoK peaked at 660 min. Significant correlations were found between KoB and KoT, KoK and KoB, and a trend for KoK and KoT during sKB. KoB significantly decreased from day 1 to day 4, then increased on day 5. KoS and KoT served as reliable proxies for KoB, while KoM exhibited differences. The study suggests non-invasive methods like tear and saliva collection can effectively measure cortisol, emphasizing the importance of calm cow handling for better welfare (110).

A study from 2021 aimed to compare eight welfare assessment protocols in relation to hair cortisol concentrations (111). Despite expectations, most protocols did not significantly correlate with hair cortisol levels, challenging the assumption that hair cortisol is a reliable indicator of cow welfare. The inconsistent correlation among protocols and their poor alignment suggests the need for further research to assess and potentially modify existing welfare assessment tools for accurate measurement (111).

The next approach aimed to pinpoint reliable indicators for assessing the well-being of dairy cows (112). The Animal Welfare and Biosecurity Evaluation form (AWB-EF), endorsed by the Italian National Center of Reference for Animal Welfare, was employed to evaluate 16 Sardinian dairy farms. Analyzing hematological parameters in 230 Holstein dairy cattle revealed a robust correlation between AWB-EF and laboratory findings. The study suggests that veterinarians can use a validated checklist alongside specific laboratory parameters to detect early signs of stress. It is noted that it is crucial to emphasize that evaluating animal welfare requires a multidisciplinary approach, and health assessment alone falls short of determining overall well-being (112).

3.10 Animal needs index

According to the substance of the following approach, the Animal Needs Index (ANI) assesses five aspects of the animal environment, including mobility, social interaction, flooring conditions, stable climate, and human care (113). They compared two organic and two conventional farms. Locomotion disorders, the first ANI category, were absent in the observed farms. The scoring considered tethered and free housing, with Farm No. 1 performing the best (85.71%) and Farm No. 3 the worst (69.2%). Cleanliness of the resting area, a part of ANI Category I, was a notable shortcoming on the farms, particularly on Farm No. 2. Social behavior, part of ANI Category II, showed unsuitable manifestations on Farm No. 3 due to tethering and lack of stable hierarchy. Bioclimate, addressing temperature and humidity, was assessed in ANI Category IV, with Farm No. 2 having the worst results. The fifth ANI category evaluated man-animal interactions and animal care, highlighting issues in farms with tethered animals (No. 2 and No. 3). ANI proved practical for welfare assessment, offering a rapid and repeatable method, but it was suggested that additional animal parameters be considered for a more comprehensive evaluation (113).

Hristov et al. (114) investigated the correlation between rearing systems, Animal Needs Index (ANI), and milk traits in five dairy farms. The rearing systems varied, with open stalls in farms A and C practicing loose cow rearing, while others tied cows in closed stalls. Outdoor pens were available in two farms. The total ANI scores ranged from A 35.5 to E 10.5, with farm A having excellent welfare levels. The rearing system significantly influenced cow welfare ($p < 0.001$) and had a notable impact on average daily milk yield, milk fat, and protein yield ($p < 0.01$). The study emphasized the importance of improving housing conditions based on ANI scores to enhance cattle production performance (114).

The authors of a paper from 2011 assessed the welfare of dairy cows in Romanian tie-stall and free-stall farms using the Austrian ANI 35 L/2000-cattle system (115). Among 40 cattle houses, free-stall barns demonstrated higher overall ANI scores compared to tie-stall barns. Welfare factors such as locomotion, social interactions, flooring, light, and air, along with stockmanship, consistently scored lower in tie-stall barns. The findings suggest that dairy cows experience better welfare in free-stall housing, highlighting the need for improvements in tie-stall barns (115).

3.11 Routine herd data and register data

In the invited review by de Vries et al. (116), the exploration of variables in routine herd data (VRHD) associated with dairy cattle welfare indicators (WI) is a key focus. Among the 27 VRHD and 34 WI under consideration, extensive associations emerged from 146 studies. Twenty-three VRHD demonstrated links to 16 WI, with

particularly noteworthy connections to milk yield, culling, and reproduction. However, limited associations were noted for WI related to behavioral aspects, disease symptoms, or resources-based indicators (116).

Another paper on this topic investigated using routine herd data (RHD) from national databases (117). Trained observers collected welfare data for 41 indicators in Dutch dairy herds, while RHD were extracted from national databases. RHD served as predictors for various welfare indicators, showing high accuracy for some. Best-performing models included indicators like access to drinkers, percentage of very lean cows, cows lying outside the supposed lying area, and cows with vulvar discharge. RHD can serve as a prescreening tool to detect herds with welfare problems, but the predictive models require validation in additional field studies (117). The same authors published a study where they used RHD and housing and management (HM) data to estimate dairy herd welfare levels more efficiently (118). The observers collected welfare data for six indicators in Dutch dairy herds, while RHD and HM data were obtained. Predictions were moderately accurate for various welfare indicators, showing potential for screening herds efficiently (118).

Register data from Nordic dairy herds, widely available for research, were assessed for their utility in identifying herds with good welfare and distinguishing between those with deficiencies (119). On-farm animal-based measurements in 55 herds formed the basis for welfare classification. A case herd with "good welfare" had no scores lying among the 10% worst in any of nine welfare measurements, with 28 herds meeting this criterion. Subsequently, 65 potential welfare indicators from a national dairy database were identified. The final set, including fertility measures, cow mortality, stillbirth rate, mastitis incidence, and feed-related diseases, showed a high sensitivity (96%) but lower specificity (56%). Combining models significantly improved welfare classification, demonstrating the use of pre-collected register data for approving dairy farms with good welfare and enhancing herd welfare assessment (119).

A study from Denmark aimed to assess register data variables as predictors of dairy herds violating animal welfare legislation (VoAWL) (120). VoAWL includes the presence of injured animals not separated or those warranting euthanasia still in the herd. Analysis of 73 Danish dairy herds identified predictors: increasing milk yield variation in first lactation cows, high bulk tank somatic cell count ($\geq$250,000 cells/ml), and a suspiciously low number of veterinary treatments ($\leq$25 treatments/100 cow years). These predictors suggest underlying management issues affecting animal welfare. Further investigations are required for causal inferences, emphasizing the need for comprehensive risk factors beyond legislative standards (120).

3.12 Cleanliness

Another study investigated the influence of cleanliness on cattle health, welfare, and farm profitability (121). In Sweden, despite legislation requiring animals to be 'clean enough,' official inspections find a significant prevalence of dirty cattle. Among 371 inspected farms, 49% had dirty cattle, but not all were considered non-compliant. The study highlights management routines as a key factor affecting cattle cleanliness. Farmers with clean cattle prioritize access to bedding material, while those with dirty cattle suggest shorter slaughter queues as a remedy. The research emphasizes the necessity for clearer guidelines in determining compliance with animal welfare legislation regarding cattle cleanliness (121).

In 2017, an Austrian dairy company introduced a third-party animal-based assessment to drive welfare improvements on farms (122). Analyzing data from 1,221 farms and 23,749 cows, prevalent welfare issues included dirty hind legs, signs of diarrhea, and hairless patches on the tarsal joint. Severe problems like very lean cows were rare. Generalized linear models revealed associations between milk delivery per cow, housing system, assessment period, and welfare outcomes. Some characteristics, however, had both positive and negative impacts, emphasizing the need for careful management to avoid undesired effects (122).

3.13 Reproduction

The effects of low and high concentrate supplementation on welfare, health, and reproduction in two dairy cow breeds on mountain farms were investigated in a study from Italy (123).

Contrary to expectations, higher concentrate levels did not necessarily result in lower animal welfare in alpine regions. One breed showed benefits with a lower calving interval and more lactations. However, caution in interpreting results is advised due to noted weaknesses in group comparison (123).

In another approach, the aim was to assess the impact of oestrus intensity and alternative indicators, such as progesterone recordings, on the reproductive performance of dairy cows (124). Results showed that heifers had a higher pregnancy rate than first-parity cows, and standing oestrus significantly increased the odds of pregnancy and calving. The eProCheck800 ELISA reader, monitoring progesterone, complemented on-farm reproductive management but had less accuracy than visual oestrus detection. Oestrus intensity was linked to good welfare, evidenced by higher pregnancy rates, emphasizing the importance of optimal oestrus expression in high-producing dairy cattle (124).

3.14 Post mortem

Knock and Carroll explored using abattoir meat inspection data to assess cattle welfare (125). They examined associations between ante-mortem issues like lameness and body condition with post-mortem measures. Results suggest recording carcass weight and bruising during meat inspection as indicators of welfare. Associations between ante-mortem indicators and post-mortem measures vary by cattle characteristics. The prevalence of bruises underscores their importance in welfare assessments. The findings propose post-mortem measures as potential indicators of cattle welfare, urging further research to establish on-farm welfare associations (125).

Another paper on this topic presents an innovative approach to retrospectively assess cattle welfare at the abattoir using claw disorders (126). The findings, based on the analysis of 1,040 cattle from various production systems, reveal a high prevalence of abnormal claw shapes and claw wall fissures. Notably, associations between lesions in front and rear limbs varied by production

system. Feedlot and free-range cattle with white line disease and skin wounds showed higher meat pH. Claw disorders serve as valuable indicators of animal fitness, reflecting their ability to cope with husbandry and pre-slaughter conditions. The importance of retrospective abattoir-level claw assessment as a tool to understand how production systems impact cattle health and welfare is pointed out. It is noted that these measures, treated as iceberg indicators, can be integrated into protocols for post-mortem cattle welfare assessment (126).

3.15 Eye white, ear posture and nasal temperature to understand cows emotions

Battini et al. (127) explored using eye white and ear posture as indicators of emotions in dairy cows. The research on five Italian dairy farms, analyzed 436 cow head photos, revealing strong correlations. Contexts like pasture access and human-animal interaction impact emotions. The study emphasizes the feasibility of on-farm assessment using photos and concludes that eye white and ear posture are valuable indicators for evaluating dairy cows' emotional well-being (127).

Another paper about ear postures as indicators of positive, low-arousal emotional states in dairy cows: Through 381 focal observations on 13 cows, four ear postures (EP1 to EP4) were analyzed during baseline, stimulus (stroking), and post-stimulus segments (128). The findings suggest that EP1 and EP2, considered relaxed postures, were more prevalent before and after stroking, while EP3 and EP4, associated with arousal, increased during stroking. These results propose that relaxed ear postures may signify positive emotional states in dairy cows. The study suggests that ear postures could serve as both immediate indicators and reflections of longer-lasting mood states in cows (128).

The use of visible eye whites as an indicator of positive emotional states in dairy cows during stroking was also investigated (129). While not currently suitable for on-farm use due to analysis time, the measure holds potential for research on emotional arousal. Further studies are needed to explore its applicability in different contexts and species (129).

A further study by Proctor et al. (130) focused on whether positive emotions affect nasal temperatures in cows. Through 350 focal observations, they induced positive emotional states in cows by stroking them. The results showed a significant decrease in nasal temperature during stroking, suggesting a change in valence. This challenges the notion that emotional fever is only associated with negative states. While nasal temperature may be a useful measure of emotional state, further research is needed (130).

3.16 Other approaches

A study from 2018 investigated the use of outcome-based observations in Assured Dairy Farm (ADF), Soil Association Organic Standards (SA), and cross compliance (CC) farm assessment reports (131). ADF reports had a higher response rate (61.0%) with resource-based comments, while SA and CC reports showed significantly more outcome-based comments. ADF comments were mainly compliant and resource-based, serving as proof of assessment. SA, emphasizing welfare outcome measures, increased outcome-based comments. CC prioritized outcome-based evidence for noncompliant decisions. The study suggests the need for a balance between general and detailed comments and proposes in-depth interviews for exploring individual rationale in future assessments (131).

In a study analyzing inspections in Swedish dairy herds from 2010 to 2013, conducted separately by the County Administrative Board (CAB) and Arla Foods, common non-compliances were identified (132). Dirty dairy cattle was a frequent issue in both systems, but substantial differences suggested distinct focuses. Risk factors for non-compliance included tie-stall housing, winter season, and, notably, overall organic farms demonstrated fewer predicted non-compliances than conventional ones (132).

An investigation by Mattiello et al. (133) aimed to compare welfare indicators among five Italian cattle breeds (Italian Holstein-Friesian, Italian Bruna, Pezzata Rossa Italiana, Grigia Alpina, and Pezzata Rossa d'Oropa) kept in tie-stalls in the Italian Alps. The study assessed integument alterations, lameness, and physical malformations in 612 cows. Results revealed a decreasing trend in welfare problems from more to less productive breeds, with local breeds exhibiting lower prevalence. Italian Holstein-Friesian generally showed the highest percentage of issues. Housing in tie-stalls was associated with welfare concerns, emphasizing the need for genetic selection changes in the dairy industry (133).

A protocol, developed for integrating herd welfare assessment into Dutch dairy farming's quality assurance program, was tested in a pilot study involving 52 herds (134). The final protocol, consisting of 16 animal-based and 14 environment-based parameters, was utilized in a voluntary field survey of 164 herds, with an average assessment time of 78 min per herd. The protocol aimed at periodic welfare auditing, emphasizing cows' biological needs. Focused on cow behavior for feasibility, the final protocol received widespread agreement among stakeholders (134).

A survey on dairy cow welfare in 7 Italian regions involved 943 farms (135). Using a checklist with 303 parameters, categorized into direct and indirect criteria covering farm management, housing, environment, feeding, and health, the study assessed animal welfare. Parameters were evaluated based on legislation and a semi-quantitative scale. Among the 249 examined, 15 had a failure prevalence below 1%, while non-compliance prevalence ranged from 2 to 67%, inversely proportional to herd size. Common non-compliance aspects related to calves management, staff training, and prophylaxis programs. Larger farms exhibited lower non-compliance, highlighting the importance of technology and staff training for better herd health. The combination of direct and indirect criteria aligns with EU animal welfare recommendations (135).

To enhance animal welfare, the Italian National Reference Center for Animal Welfare (CReNBA) promotes 38 best practices for dairy cattle (136). Covering managerial and equipment factors, these practices shift towards "positive animal welfare" (PAW), considering a life worth living. CReNBA's welfare assessment protocol, part of the "ClassyFarm" system, incorporates hazards and benefits for a comprehensive guide (136).

A study by Pezzuolo et al. (137) introduces a cost-effective 3D camera system for frequent growth assessment of calves and cows. Verified for accuracy through uncertainty analysis and calibration, the system showed generally precise measurements, with deviations under

6% compared to manual measurements, except for specific parameters. With increasing stock densities on dairy farms, the non-contact measurement approach becomes valuable (137).

In a survey involving 16 Italian veterinarians, a Delphi technique was used to assess hazards and welfare promoters in loose housing systems for dairy cows (138). Hazards affecting lactating cows, such as inadequate flooring and lack of bedding, were rated high. Welfare promoters, including optimal resting conditions and skilled stockpersons, received top ratings. Animal-based measures like lameness observation and mortality records were considered crucial (138).

A paper by Katzenberger et al. (139) assessed the feasibility of farmers' self-assessment for a dairy cattle welfare assurance program in South Tyrol. The inter-rater reliability between experts and farmers in assessing welfare outcomes was found to be slight to moderate (139).

4 Discussion/conclusion

This comprehensive systematic review accentuates the pivotal role of the WQ® protocol in evaluating dairy cattle welfare, acknowledging its versatility in identifying risk factors and assessing various parameters. Despite its acknowledged effectiveness, challenges like time consumption persist, prompting ongoing innovative efforts for protocol refinement and alternative assessment methods. Benchmarking, exemplified in diverse welfare assessment protocols, serves as an important tool for targeted improvements and overall welfare enhancement. Correlations between lameness, mobility scores, and adverse effects on production underscore the need for early identification through technology. Dairy cow behavior analysis provides valuable insights into their well-being, emphasizing the importance of understanding and enhancing welfare through various measures.

Exploring the human-animal relationship in dairy farming is pivotal for ethical considerations and welfare choices. Housing and pasture systems significantly impact dairy cow welfare and productivity, with studies favoring pasture-based systems. The DairyCheck sensor system, monitoring chewing muscle activity, showcases promising capabilities for personalized evaluations and early ailment detection. Heart rate and heart rate variability monitoring offer valuable insights into welfare, with automated milking systems presenting potential advantages. Biomarkers play an essential role in balancing productivity and health, as shown in the DairyCare project. Cortisol is a promising biomarker for assessing dairy cow welfare due to its ability to effectively reflect stress levels. It can be measured non-invasively in methods such as saliva, tears, and feces, minimizing stress on the animals during sampling. Further research should continue in this direction to enhance understanding and application.

Precision Livestock Farming offers real-time welfare assessment, but validation is important. Monitoring vocalization, correlating milk yield with well-being, and assessing bulk tank milk data reveal complex relationships between productivity, emotional experiences, and overall welfare. Stress physiology is multifaceted, influenced by factors like resting comfort, human-animal contact, and feeding practices. A multidisciplinary approach provides a comprehensive understanding of early signs of stress and contributes to overall well-being assessment in dairy cows.

The Animal Needs Index offers a rapid method for assessing dairy cow welfare, emphasizing the influence of rearing systems. Routine herd data analysis reveals significant links with milk yield, culling, and reproduction, aiding in prescreening for potential welfare concerns. Predictors of dairy herds violating animal welfare legislation underscore the importance of comprehensive risk factors. Cleanliness emerges as a relevant factor in cattle management, impacting health, welfare, and farm profitability. Unexpected outcomes in concentrate supplementation caution against simplistic interpretations, while optimal oestrus expression proves vital for reproductive performance.

Diverse approaches, including abattoir data analysis, claw disorders, visual indicators of emotions, and innovative technologies, contribute valuable insights into cattle welfare assessment. The integration of outcome-based observations, breed-specific considerations, and the development of practical protocols and technologies further advance our understanding and ability to enhance dairy cattle welfare across various farming systems.

Commercial animal welfare audits must rely either on easily observable well-being indicators or on information from herd records. The ability to measure biomarkers or heart rate variability during an audit is limited due to several practical and logistical reasons. Measuring biomarkers and monitoring heart rate variability require specialized equipment and expertise, which are often expensive and not easily portable for use during an audit. Additionally, the analysis and interpretation of the results require time and expertise, which may not always be available during a standard animal welfare audit on a farm. Efficiency and time-effectiveness are important for animal welfare assessments, especially considering the limited time available for audits and the potential slowdown caused by complex measurement methods.

For these reasons, easily observable well-being indicators such as BCS, lameness, claw health, cleanliness, and somatic cell count provide practical and readily accessible data. These can be assessed without special equipment, making them ideal for use during an audit. Additionally, information from herd records can offer valuable insights into animal well-being, including feed rations, health treatments, reproductive data, and milk production. These data are often well-documented and easily accessible, facilitating their integration into animal welfare audits.

The information gained from this systematic review can seamlessly integrate into existing commercial animal welfare assessments. Indicators such as BCS, lameness, claw health, and cleanliness offer practical and measurable criteria that can be easily incorporated into routine assessments. This allows farmers and auditors to promptly respond to potential issues and take targeted actions to improve animal well-being.

Further research into abbreviated protocols, such as the DCF protocol, would be beneficial. The DCF protocol showed correlations with the WQ® protocol while requiring significantly less time, suggesting that streamlined approaches could offer practical alternatives without compromising assessment quality. It saves

approximately 6 h on a farm with 200 dairy cows, making it much more feasible for regular assessments. This is more beneficial for farmers balancing numerous daily tasks, ensuring that welfare evaluations can be integrated into routine operations without major disruptions.

The DCF protocol relies on simpler, more direct indicators that are quicker and easier to assess, such as BCS, lameness, and cleanliness. These animal-based measures provide immediate feedback and are practical to evaluate during routine checks. In contrast, the WQ® protocol includes more complex evaluations that can be time-consuming and require specialized training.

Additionally, the DCF protocol uses a more transparent and straightforward method of summarizing welfare measures. Unlike the WQ® protocol's complex weighting and aggregation methods, the DCF protocol's summarization is easier to understand and implement, ensuring that farmers can readily interpret and act on the results.

Furthermore, it requires inspecting fewer cows (16% of the population) compared to the WQ® protocol (38%), contributing to its time efficiency and practicality without significantly compromising accuracy.

In summary, the design of the DCF protocol makes it a more suitable tool for everyday use by farmers and in commercial animal welfare audits. Given these promising results, further studies should be conducted in this direction to gather more data on the DCF protocol. This would enable the direct use of the DCF protocol itself or the development of a similar standardized protocol that is comparably accurate to the WQ® protocol. Establishing a protocol from existing validated indicators that, when combined, offer a comprehensive and objective overview of the welfare status of cows would facilitate standardized and easily comparable assessments of animal welfare.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Author contributions

JL: Writing – original draft, Data curation, Formal analysis, Investigation, Methodology, Visualization. CT-R: Writing – review & editing, Conceptualization, Methodology, Project administration, Supervision. RM: Writing – review & editing, Conceptualization, Methodology, Project administration, Supervision.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

We thank Maiken Mondon for the preliminary literature search.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

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frontiers | Frontiers in Veterinary Science

TYPE Original Research
PUBLISHED 07 August 2024
DOI 10.3389/fvets.2024.1442580

OPEN ACCESS

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RECEIVED 02 June 2024
ACCEPTED 23 July 2024
PUBLISHED 07 August 2024

CITATION
Rokade J, Champati A, Sonale N, Wadajkar P, Madheshwaran M, Bhaisare D and Tiwari AK (2024) The cage-free egg sector: perspectives of Indian poultry producers. *Front. Vet. Sci.* 11:1442580. doi: 10.3389/fvets.2024.1442580

The cage-free egg sector: perspectives of Indian poultry producers

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India is ranked as the 2nd largest egg producer in the world. Despite the prevalence of backyard poultry (free range), a majority of the commercial egg-laying hens in the country are still housed in battery cages. There is a global shift toward cage-free eggs, due to regulations and increased demand from conscious consumers and food corporations. However, there are very few commercial cage-free facilities in India to meet this demand. The aim of this study was to undertake a needs-assessment survey of Indian egg producers on cage-free production, and understand what support is needed to build the capacities of the cage-free egg production sector to develop it into a viable and sustainable alternative to battery cage eggs. The results showed that nearly all producers agreed on the need for additional support in shifting to, and operating in, the cage-free sector. This included support in the form of financial assistance, technical training, and promotion of the cage-free sector. The results of this study highlight the pressing need for government and private support, in the absence of which cage-free producers are compelled to compete with battery cage poultry producers on prices, which will result in increased losses and failure of the sector, since they have not yet achieved economies of scale.

KEYWORDS

cage free, egg, welfare, layer, chicken

1 Introduction

Asia has been a global leader in egg production for decades, contributing to approximately 60% of the global production. In 2018, annual egg production in the region was 822 billion, through 3.1 billion layer hens (1). India has also seen tremendous growth, producing 138.38 billion eggs in 2022–2023, with the poultry market valued at INR 1905.3 billion, making India the 2nd largest egg producer in the world (1, 2). The sector is projected to continue its growth in the coming years, and is expected to reach INR 3477.8 billion by 2028 (3).

Much like the rest of the continent, layer hens (*Gallus domesticus*) used in the egg industry are housed in battery cages, i.e., barren wire-mesh cages housing 4–5 birds per cage. Battery cages have been widely challenged as cruel systems that provide inadequate housing for hens due to a lack of necessary space for movement, species-inappropriate flooring, and lack of opportunities to express natural behaviors, leading to physical and psychological suffering (4, 5). Recognition of the cruelty inherent to battery cages has also resulted in government bans

on these barren cages, as seen in the European Union and several US states (6–8). Cage-free housing is recognized for providing better welfare due to increased space availability per bird and enrichment material and opportunities that facilitate the expression of natural behavior (9).

An increasing number of studies have documented a shift in consumer preferences toward higher welfare food products such as cage-free eggs, which ensure better living conditions for the animals involved (10–12). A growing concern for ethical consumption has also resulted in a shift in institutional consumption patterns, with thousands of food corporations, including those located in India, committing to use only cage-free eggs within the decade (13).

While corporations have made progress toward this goal in countries in the Global North, the rest of the world, particularly Asia, has seen very little progress toward cage-free procurement (14, 15). As of 2023, the Asia-Pacific (APAC) region has been found to have an average transition of 57% (16, 17).

This is, in large part, due to the infancy of the cage-free sector in Asia, and more specifically India. Most layer hens in India are still housed in battery cages, despite the prevalence of backyard poultry (free-range), particularly in periurban and rural areas (18). However, these poultry operate on a very small scale, with the eggs being consumed by the producers themselves or supplied to a few local families. There are very few cage-free farms that operate on a commercial level, as an alternative to the conventional battery cage facilities that are a prevalent practice in the country. The primary causes of the poor popularity of cage-free egg production in India are: lack of public and farmer awareness; an unorganized Indian market for cage-free eggs; a lack of technical information or HRD support; a lack of accountability for separating cage-free from non-cage eggs; Absence of government initiative, particularly in export assistance and market regulation. Given the increasing demand for cage-free eggs from conscious individuals and institutional consumers who are moving toward ethical sourcing, there is a pressing need for the growth of the Indian cage-free sector (3).

This study was undertaken to understand the perspectives of egg producers on the issue of cage-free systems, the key challenges in shifting to and operating in this sector, and the solutions and support required to overcome these barriers. The main goal was to understand the support needed to build the capacities of the cage-free egg sector in order to develop it into a viable and sustainable alternative for egg production in India.

2 Materials and method

2.1 Participants

Respondents were interviewed in October 2023 across the states of Karnataka, Telangana, Tamil Nadu, Andhra Pradesh, Uttar Pradesh, and Madhya Pradesh. The interviews were also recorded with the consent of the participants. The states covered in this study were selected on the basis of convenience sampling. Participants were approached by local collaborators who were familiar with the project topic, were given training in data collection, and briefed prior to conducting interviews. Since the cage-free sector in India is in its infancy, there are a limited number of producers who operate commercial cage-free facilities, limiting the sample size for the survey. Twenty egg producers across the country were engaged in this study. Out of these, 10 operate cage systems and 10 cage-free systems for egg production. The capacity of the farm, i.e., the number of laying hens reared by the respondents of the study included 50% having under 10,000 birds capacity; followed by 30% ranging 10,000–20,000; 10% with 20,000–50,000; and 5% each of 50,000–100,000 and above 100,000. It was seen recorded that the majority (70%) of the cage-free farmers had a capacity of under 10,000 birds with maximum cap of 20,000, whereas the cage farming respondents capacity ranged from under 10,000 to even over 100,000 bird capacity.

Participants were eligible to participate in the study if they:

- Gave their consent in writing, which was included on the questionnaire,
- engaged in commercial egg production,
- worked in the industry for at least 1 year, and
- operated in a managerial or ownership position at the facility.

2.2 Research tool

A combination of qualitative and quantitative methods were adopted for data collection and analysis. Separate questionnaires were designed for cage and cage-free producers. Kobo toolbox was used to create the questionnaire in English and translators were employed to convert the questionnaire in local languages- Hindi and Kannada. The answers were also collected using Kobo toolbox at each egg producing unit, through a network of local collaborators fluent in the respective native language. Responses in Hindi and Kannada were translated to English while filling the forms. The questionnaire consisted of 14–23 questions, as certain questions had follow-ups that would only apply if a specific answer was provided, excluding the producer's contact details, facility name, and geographical location.

In this survey, we defined *cage-free farming* as a method of raising hens in non-caged housing, providing them with the freedom to move, stretch their wings and ideally access nest boxes, perches, foraging areas, and dust bathing spots. *Cage farming,* on the other hand, refers to the method of confining hens in small wire cages, typically in large numbers, where they are unable to exhibit their natural behaviors.

The questions that are relevant for our purposes are as follows:

1 *Most egg farmers in our country and around the world use cages. What are the reasons for using cages compared to cage-free systems? (Open-ended)*
2 *Some egg farmers are changing to cage-free systems. What do you think are the reasons to use cage-free rather than cage systems? (Open-ended)*
3 *What do you think are the biggest challenges and problems that prevent cage farmers from using cage-free systems? (Open-ended)*
4 *If an egg farmer decided to use a cage-free system what would be some of the solutions to the challenges outlined in the question above? (Open-ended)*
5 *If an egg farmer decided to use a cage-free system, would they need more support in the establishment or maintenance of the farm than is currently available? (Yes/No)*
6 *What support would they need? (Open-ended)*
7 *Who should offer that support? (Open-ended)*
8 *What are the main operational challenges in running your cage-free farm? (Open-ended)*

2.3 Data analysis

The data collected was analyzed using thematic qualitative analysis and descriptive quantitative statistics. All available responses were included in the analysis. Numerical data was analyzed using Microsoft Office tools.

3 Results

This study looks at the responses of egg producers to better understand what producers operating cage facilities perceive as the biggest challenges in shifting to cage-free systems, the real challenges experienced by those engaged in cage-free production, and the solution and support required to overcome the identified barriers. In seeking an answer to what support is required to build the capacities of the cage-free egg production sector to develop it into a viable and sustainable alternative to battery caged eggs, the results have been categorized into five themes:

1 Advantages of battery cage facilities;
2 Reasons to adopt cage-free systems;
3 Challenges in cage-free systems;
4 Potential solutions to challenges identified in cage-free systems; and
5 Support needed to transition to cage-free systems.

3.1 Advantages of battery cage facilities

> *"Most egg farmers in our country and around the world use cages. What are the reasons for using cages compared to cage-free systems? (Open-ended)".*

The most cited reason for preference for battery cage facilities is the ease in management of these facilities, in terms of providing vaccines and medication, maintaining biosecurity and controlling diseases, feeding, and egg collection. Lower costs of production was an additional factor for choosing caged systems. All responses are displayed in Table 1.

TABLE 1 Reasons stated for producers choosing caged systems over cage-free systems.

Theme identified	Factors	*n*	Percentage of responses
Economic considerations (33.33%)	Higher egg production	1	3.3
	Higher demand	3	10.0
	Industrial push toward cages	1	3.3
	Reduced egg breakage	2	6.7
	Automated feeding and watering	3	10.0
Health/Disease (33.33%)	Cleaner eggs	3	10.0
	Ease of medicines and vaccinations	3	10.0
	Reduced disease transmission	4	13.3
Investment required in caged production (26.67%)	Less space requirement	4	13.3
	Lower cost of production	1	3.3
	Less labor intensive	3	10.0
Hygiene concerns (6.67%)	Cleanliness	2	6.7

3.2 Reasons to adopt cage-free systems

> *"Some egg farmers are changing to cage-free systems. What do you think are the reasons to use cage-free rather than cage systems? (Open-ended)"*

3.2.1 Battery cage producers

Respondents operating battery cage facilities highlighted cost as a major factor in considering cage-free over cage systems. High infrastructure costs can make the establishment of battery cage facilities prohibitively expensive, taking into account the cost of the cages themselves, which one respondent shared was around Rs. 8–9 lakhs to house 5,000–6,000 birds. They also shared that when battery cage suppliers are located in other parts of the country, transportation costs add to the large investment required to set up these facilities. Wear and tear of the cages is also a cost addition, necessitating a replacement every 10–15 years. In comparison, cage-free facilities are a lot cheaper to establish.

3.2.2 Cage-free producers

The primary reasons that respondents cited for opting for cage-free facilities are the increased welfare of the layer hens, making this a more humane form of egg production, followed by the growing cage-free sector in the country. Respondents also preferred the ability to get higher and consistent prices year-round, as well as autonomy in deciding prices, as they are not dependent on the external parties. Some responses also highlighted the lower dependence on antibiotics and higher quality and nutrition of cage-free eggs. All the responses are displayed in Figure 1.

3.3 Challenges in cage-free systems

3.3.1 Battery cage producers

> *"What do you think are the biggest challenges and problems that prevent cage farmers from using cage-free systems?"*

When asked about the perceived challenges that may prevent the transition to cage-free systems, respondents operating cage systems were mainly concerned about the higher cost of production due to the presumption that cage-free facilities are more labor intensive and require a lot of land. They also cited greater challenges in feeding, watering, vaccination, medication and management of hens in cage-free facilities. Other concerns involved management, training and awareness, as well as the fear of lack of demand for cage-free eggs.

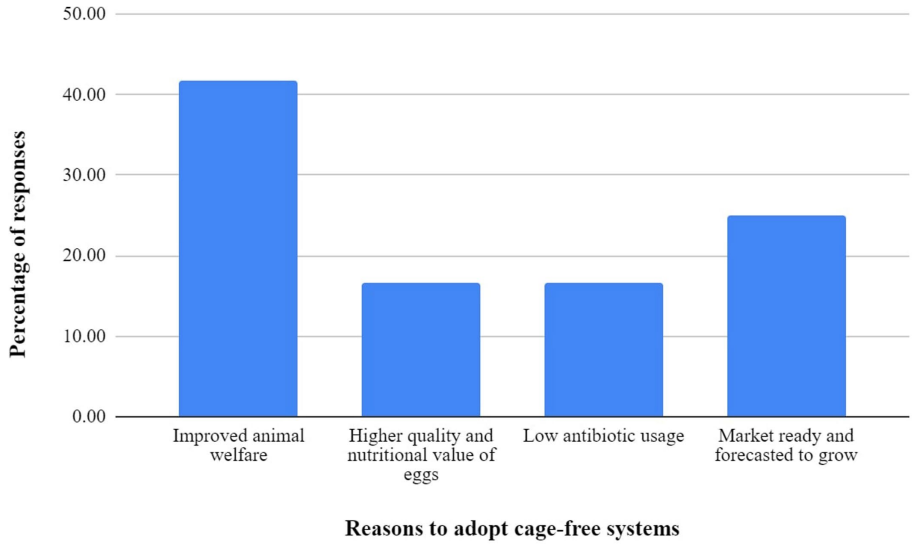

FIGURE 1
Responses of cage and cage-free producers regarding the rise of cage-free facilities.

TABLE 2 Ranking of the challenges perceived by battery cage producers in cage-free facilities.

Theme	*n*
High disease transmission	4
Frequent broken eggs	3
Difficult to monitor	3
Difficulty collecting eggs	3
Unclean eggs	3
Predator attacks	3
Space constraints	3
Difficult to vaccinate	2
Inadequate demand	1
High cost of production	1
Inadequate profits	2
Labor intensive	2
No precedent of large scale commercial cage-free farming	1

Multiple respondents indicated the need to see a successful cage-free facility before considering a transition.

Many respondents also confused cage-free facilities with free range poultry facilities and believe hens to be vulnerable to animal attacks and diseases from migratory birds. All the responses are portrayed in Table 2.

3.3.2 Cage-free producers

> *"What are the main operational challenges in running your cage-free farm"?*

The most pressing challenges identified were an increased resource dependence, including more labor and higher feed consumption. Lack of training in farm management for welfare and disease prevention was also a pressing concern. Additionally, a lack of awareness and market understanding about cage-free eggs is another challenge they faced. All the responses are portrayed in Table 3.

3.4 Potential solutions to challenges identified in cage-free systems

> *"If an egg farmer decided to use a cage-free system, what would be some of the solutions to the challenges outlined in the question above? (Open-ended)".*

The primary solutions identified to address the perceived challenges in operating cage-free systems included better training of staff, increased government assistance, improving consumer awareness, and better farm management practices. Respondents also highlighted the need to see successful large-scale cage-free facilities to fully understand how they operate and earn profits. All the responses are portrayed as an aggregate in Table 4.

3.5 Support needed to transition to cage-free systems

a *If an egg farmer decided to use a cage-free system, would they need more support in the establishment or maintenance of the farm than is currently available? (Yes/No)*

The overwhelming response regarding the need for increased support for cage-free facilities was in the affirmative. This held true across both battery cage and cage-free respondents. Responses are displayed in Figure 2.

TABLE 3 Ranking of the main operational challenges faced by cage-free producers.

Theme	*n*
Large scale unviability	1
Low demand	1
Unclean and broken eggs	1
High space requirement	2
Lack of training	3
Labor intensive	3
High cost of production	3
Disease outbreaks	3

TABLE 4 Suggested solutions to the challenges faced by egg producers in cage-free systems.

Theme	*n*
Employing more trained staff	1
Using nest boxes to prevent broken eggs	1
Litter management to prevent disease	1
Barricades (in free range)	1
Assistance from government	1
Farm monitoring	2
Preventative medication	2

b *What support would they need? (Open-ended)*

Respondents identified financial assistance as the most important support to establish or operate cage-free facilities. Increased technical support in the form of training in management practices, market support through increased awareness, and uniform standards through certification were also highlighted. Responses are displayed in Figures 3, 4.

c *Who should offer that support? (Open-ended)*

Both cage and cage-free respondents primarily identified the government as the body to offer increased support to establish and manage cage-free facilities. Some respondents also shared the need for support from other parties, such as banks, established poultry players, and poultry associations. All responses are displayed in Figures 5, 6.

4 Discussion

4.1 Reasons to adopt cage-free systems

The findings of this study present efficiency as the primary reason egg producers opt for cage systems, i.e., it is easier to provide feed, vaccinations and medication, and prevent diseases, with lower labor and land requirements. In the artificial conditions of cages, eggs are cleaner, less prone to breakage, and easier to collect.

However, high establishment costs are a major challenge to setting up caged facilities. The large expenses required in procuring cages, as well as the need to replace them every 10–15 years, was identified as a reason to opt for cage-free systems. A study found that although cage-free systems may potentially reduce profitability (19), it was made up with the possible cost savings from not requiring cage installations (20).

The present study found that caged egg producers in India are open to a possible transition to cage-free systems, and acknowledge certain advantages as well, but are largely constrained by a lack of available support. This is in line with past studies where producers have acknowledged the feasibility of cage-free transitions in other parts of Asia (20, 21). Respondents in this study also expressed not being confident about the commercial viability of cage-free systems due to an absence of large, profitable cage-free ventures to refer to as examples.

The primary reason stated for adopting cage-free systems was better animal welfare. It is widely recognized that there is an improvement in the welfare of laying hens in non-caged systems, in terms of both physical and psychological benefits (4, 22). Studies have also documented the reduction of pain suffered by hens in cage-free systems when compared to battery cage systems, finding that disabling pain is reduced by 63%, hurtful pain by 57%, and annoying pain by 70% (23).

Other driving factors were the increased consumer demand through corporate commitments, improved quality of eggs, and decreased use of antibiotics. Respondents operating cage-free systems highlighted better prices as an additional advantage, since they enjoy consistent prices throughout the year, and autonomy in deciding egg prices, with no dependence on external parties. All these benefits are a result of the continued growth of the cage-free sector in India, through increased consumer awareness, and demand at an individual and institutional level. A study conducted in 2022 highlighted a global increase in consumer awareness and concern for animal welfare in food production systems, with 71.9% of consumers from India agreeing that battery cage systems are cruel (12).

4.2 Challenges in adopting cage-free systems

In this study, the constraints perceived by cage producers in moving to cage-free systems include a higher cost of production, lack of awareness and training, increased land and labor requirement, and reduced profitability due to low demand. The other challenges listed were higher risks of disease outbreaks, difficulty in monitoring and record keeping, and higher incidence of unclean and broken eggs. A lack of awareness regarding the difference between free range/backyard poultries and cage-free systems also led to concerns about risk of attacks from predators, and spread of diseases from migratory birds.

In comparison, responses from cage-free producers also highlighted the issue of higher costs, attributed largely to increased feed consumption by birds that are allowed the freedom to move. However, they recognized that there is a growing demand for cage-free eggs by both individual and institutional consumers, countering perceived concerns about reduced profitability. This aligns with findings from other studies. Globally, there has been a rise in cage-free egg production following the 2012 EU ban directive (22), due to pressure from consumers regarding the welfare of layer hens. Consumers around the world support higher welfare eggs in the face of the cruelty experienced in caged facilities. A survey in Asian

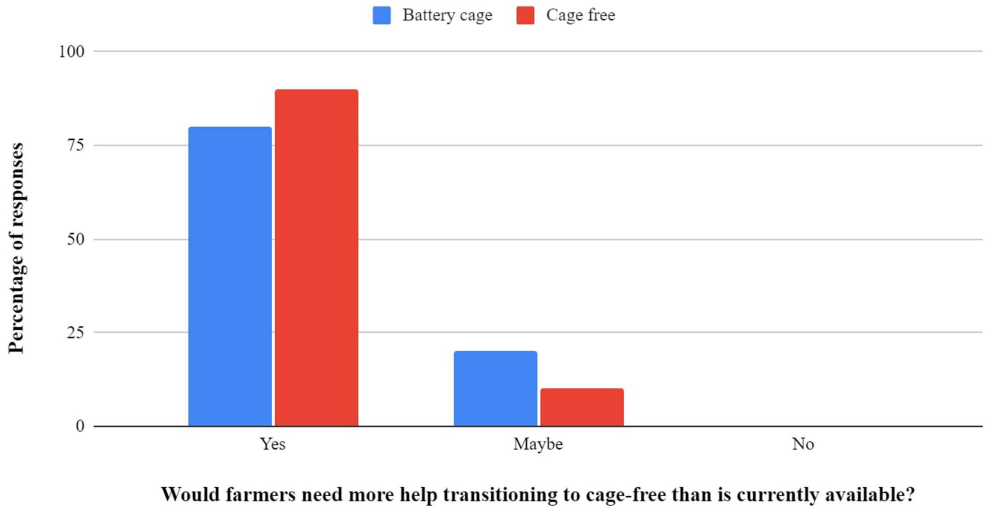

FIGURE 2
Cage and cage-free farmer's responses to whether support is required for the establishment and maintenance of a cage-free farm.

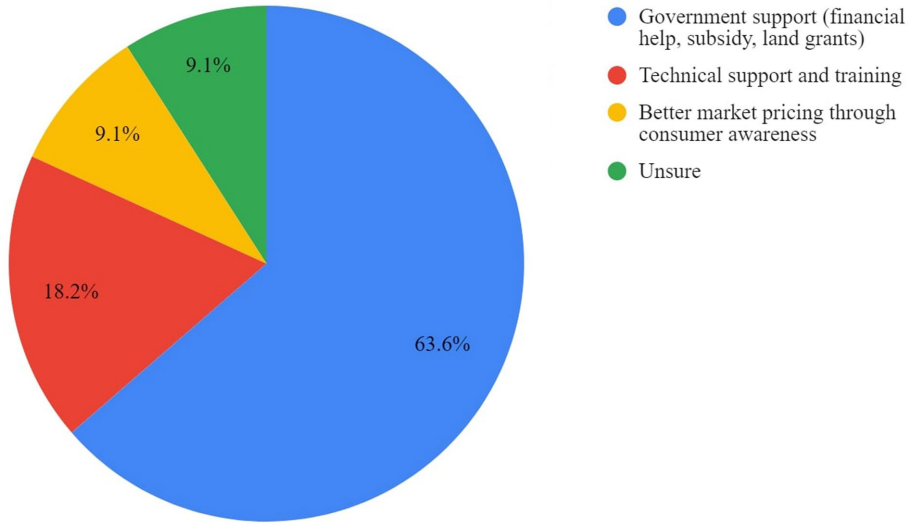

FIGURE 3
Support needed to transition to cage-free farming as perceived by cage producers.

countries (20), stated 70–80% of egg consumers preferred that hens not suffer, and about 65–70% preferred cage-free eggs (12). The rising demand for cage-free eggs is evidenced through commitments made by food corporations to switch to higher welfare eggs within the decade (9).

Cage-free respondents aligned with caged producers on concerns about increased labor requirements, attributing it to a lack of automated systems in India's nascent cage-free sector. Disease prevention was another common concern raised by respondents from both systems.

Aside from the above challenges, cage-free respondents were found to be deeply concerned about the lack of support structures for the sector, such as training opportunities, financial support, governmental recognition, and certification standards, which hinder their ability to operate and expand their scale of operations.

Other challenges envisioned by the caged producers regarding record-keeping, cleanliness, damaged eggs and unwanted behavior can be addressed through better management practices. Such problems were not faced by cage-free respondents in the current study, nor producers in other studies (20). Finally, one of the highlighted barriers raised by caged producers in the current study was a lack of land or space availability for shifting to cage-free production, which coincides with findings from other studies as well (20).

4.3 Solutions to the challenges

The main challenges in cage-free systems, as shared by respondents in the present study, were reduced profitability, higher production costs, disease outbreaks, and inadequate knowledge. These

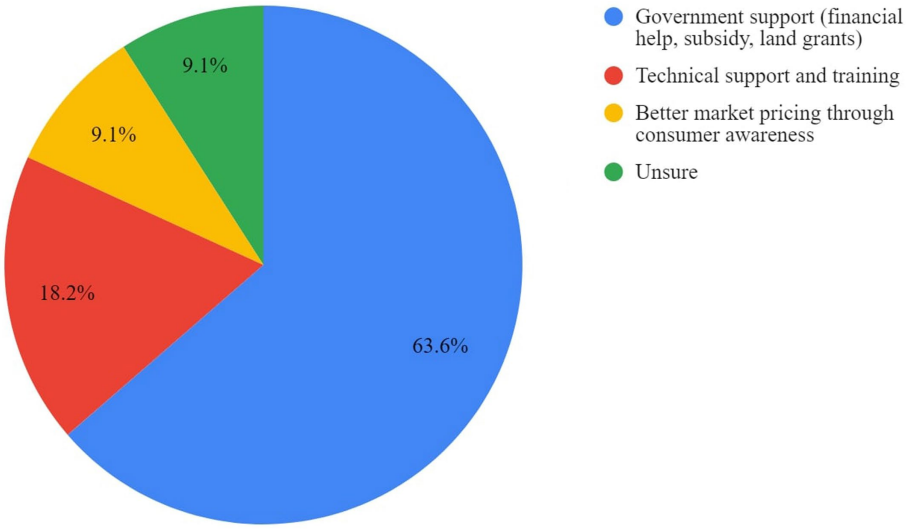

FIGURE 4
Support needed to transition to cage-free farming as perceived by cage-free producers.

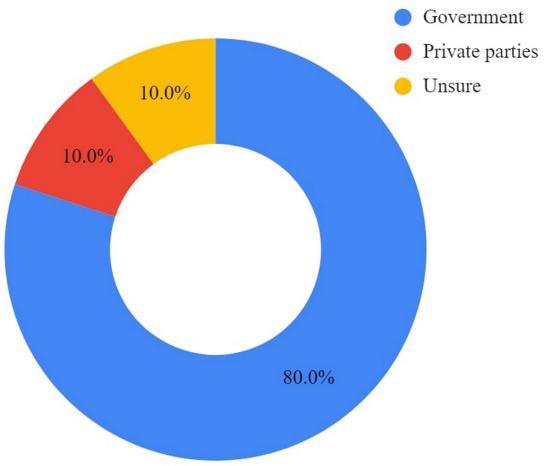

FIGURE 5
Cage farmers' perspectives on who should offer support in transitioning to cage-free housing systems.

conclusions are similar to other studies conducted around the world (20, 24–26).

When asked for possible solutions to these challenges, respondents listed training opportunities and material, government support for finances, certification and labeling, and development of markets through awareness and education initiatives. These findings were in corroboration with other studies in Asia (10, 20, 27), in which egg producers in cage systems suggested that an increase in the sale of higher welfare eggs, along with supplementary measures like training in cage-free management and proper regulations, would lead to a smooth transition into producing cage-free eggs for a sustainable future (27). The need for increased public awareness to shift consumer preferences toward higher welfare eggs like cage-free has also been recognized, as there is low awareness about the conditions of laying hens in the egg industry (24).

Concerns raised by cage producers over unclean/broken eggs, difficulties in handling birds and preventing feather pecking, which were not corroborated by those engaged in the cage-free sector, can be addressed by better management (20). Improved farm management, facilitated through training programs and material, can address these issues. For instance, concerns about unclean and broken eggs are addressed by cage-free producers by utilizing nest boxes. Concerns about feather pecking are addressed by adding enrichment, such as pecking material, to the facility.

Cage-free housing is not in itself a cause for higher incidence of diseases. On the contrary, a report by the European Food Safety Authority found that caged production systems have a higher prevalence of salmonella, compared to non-caged systems (28). Effective management practices, such as monitoring the birds' health, and timely administration of vaccines and medication can address concerns about disease prevention.

An additional challenge is the lack of adequate land availability which is a concern not just for participants of this study, but others as well. A transition to cage-free housing requires more space for each bird, which is a large factor in moving away from caged systems. However, establishing multi-level aviaries can address this issue - by increasing the number of hens housed in a given area, while maintaining adequate space requirements and welfare provisions (22, 31).

In the present study, some respondents could not offer any solutions to the barriers identified, and shared that the absence of success stories about large-scale commercially viable cage-free facilities makes it hard to envision such operations. There was a similar finding in another study (20, 27) where the respondents gave similar inconsistent responses in relation to commercial cage-free egg production. This highlights the need for model cage-free systems on a commercial scale, where producers can receive training on better management practices to improve productivity, welfare, and profitability. Additionally, these model facilities can also establish effective biosecurity measures to reduce disease prevention, and share the latest technologies and strategies to

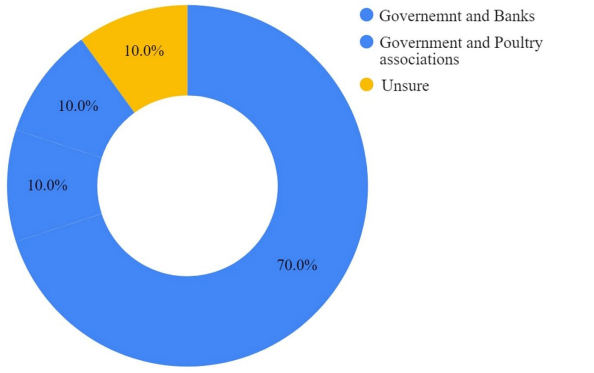

FIGURE 6
Cage-free farmers' perspectives on who should offer support in transitioning to cage-free housing systems.

operate and manage cage-free facilities in a way that meets welfare and profit requirements.

4.4 Support needed in cage-free systems

In the present study, nearly all respondents across both categories believed that some form of support was necessary for establishing and operating cage-free systems. This can be broadly divided into-financial support, technical know-how, market support, and improved consumer awareness.

When asked who should provide such support, a majority of the respondents identified the government to provide support in terms of financial assistance, training and technical support, recognition and promotion of the cage-free sector, and introduction of standards for certification and labeling. Other sources of support were identified, such as banks (for financial support through low-interest loans, for instance) and poultry associations (for technical support such as management handbooks).

These findings align with past studies. One found that respondents pointed to support required from the government in terms of finance, training and extending awareness among consumers (29, 30). In another study, stakeholders reported increased consumer awareness and producer training as solutions for increased welfare-based egg production (30).

4.5 Applications

The findings of this study help in understanding the rationale behind the producers' decisions toward adopting specific housing systems, the barriers in moving to (and operating) higher welfare systems, and the solutions required to do so. Through their input, this study has identified the need for financial support as well as its forms and sources; the gaps in technical knowledge on cage-free production methods and how they need to be bridged; and the market support required to ensure growth in this sector.

Input from stakeholders directly engaged in egg production is essential for informed decision-making regarding the promotion of higher welfare egg production. Accordingly, this study can serve as a resource to

- better understand the cage-free sector and its current limitations;
- understand the needs of egg producers when considering a shift to higher welfare forms of production;
- understand the support required to manage and grow existing cage-free operations;
- make policy decisions to support the cage-free sector in the country; and
- develop systems and materials to share technical knowledge;

4.6 Suggested initiatives

In light of the challenges, solutions, and forms of support shared by participants, as well as an analysis of other papers and studies, the following initiatives are likely to help the Indian cage-free sector grow:

- Development of management guide – Housing (for successful farming- Nine birds per sq. meter), nutrition, management details must be provided.
- Increased governmental financial support for cage-free production, in the form of subsidies, schemes, incentives, ease of business, and low interest loans.
- Certification and labeling standards for cage-free systems.
- Model facilities to provide on-site training for producers, and showcase the commercial feasibility of large-scale cage-free egg production.
- Increased technical support through training programs, manuals, and guides, and sharing technological advancements to improve management practices.
- Increased research to develop models, technologies, and methods to improve the efficiency of cage-free practices to make them more commercially feasible.
- Awareness programs to educate consumers and producers about cage-free systems, a sustainable and higher welfare model of egg production.

According on earlier research cites (20, 32), the following initiatives are recommended in the study to assist the Indian chicken industry in transitioning to sustainable, cage-free production:

Increase knowledge of the realities of effectively managed cage-free systems within the egg industry. On a big commercial basis. Encourage cooperation between local governments and egg producers in order to find appropriate land parcels for the pilot program of cage-free growing. Boost awareness and education about cage-free systems by creating training programs on best practices for managing them and inviting important stakeholders to participate. Pay particular attention to food safety, biosecurity, and efficient disease mitigation techniques.

5 Limitations

A negligible number of studies have been conducted in India analyzing cage and cage-free egg production. This study presents a starting point to conduct further in-depth research into this sector.

A significant limitation of this study is the small sample size, owing to the limited number of commercial cage-free facilities in the country, as this sector is in a nascent stage. Maintaining an even split between cage and cage-free respondents limited the sample size accordingly. However, the study provides insights into both real and perceived challenges regarding cage-free production, and the support required to overcome them. Another limitation is the large variability in the size of the poultry facility (2,200 to 1.35 lakh birds).

This study and the limitations therein highlight the need for an additional comprehensive exploration of the cage-free sector in India, and the needs of the producers and consumers.

6 Summary of economics

- Cage-free egg production is an intensive system of rearing as the farmers in Indian is following stocking density range from 11 to 12 birds/m^2 for pullets and 9 to 10 birds/m^2 for adult birds.
- At international level ideal stocking density should be 6.17 birds/m^2 (European legislation), 7.15 birds per m^2 (Global Animal Partnership, 2017), 6.2 birds per m2 (AGW Animal Welfare Approved), The German label "Für mehr Tierschutz" (standard and premium) allows a maximum stocking density of 7 birds/m^2.
- It is different farming from the basic backyard farming where we gave access of foraging to the birds but in Cage Free rearing birds are stall fed for lifetime without any opportunity of foraging. It's nothing but commercial intensive layer farming in deep litter with enrichment facilities, and we should not confuse with Backyard small scale farming.
- The Cage free farmers participated in our survey have minimum of 2,220 birds and maximum 20,000 bird's capacity farm. We strongly feel for good commercial output minimum of 1,000 birds stock is needed
- Based upon our study we have come up with following model of 1,000 birds-

1. Birds capacity- 1000
2. Average efficiency of production – up to 92%
3. Average egg production of birds- 240-260 eggs per year
4. Mortality- 7-10%
5. Cost of Production of one egg- 6.5 Indian rupees per egg (including recurring and non-recurring cost)
6. The cost of packaging, storage and transport per egg- 0.5 to 0.7 Indian Paisa
7. The cost of Sale- 12-20 Indian rupees per egg depending upon branding.
8. Pure Profit per egg - Varying from 3 to 7 Indian rupees per egg

7 Conclusion

As consumers in the Indian sub-continent increasingly prioritize ethical considerations in their purchasing decisions, the egg production industry needs to adapt to meet these evolving expectations. The transition to cage-free production represents a significant stride toward creating a more ethical and sustainable future for egg production. While acknowledging the positive aspects of cage-free production, it is essential to recognize the challenges associated with this transition. A significant insight that emerged from this study was that in the absence of adequate aid from the government, cage-free producers are compelled to compete with battery cage poultry producers on prices, which will result in increased losses and failure of the sector.

The current study is aimed at understanding the reasons and challenges in considering the adoption of cage-free systems. The possible solutions and types of support were also discussed, and applications were suggested on the basis of the results. The exploration of cage-free production in India underscores the interconnectedness of animal welfare, industry sustainability, and consumer preferences. While the challenges are significant, they are likely to be addressed as technology, research, and industry expertise continue to advance. This will pave the way for more widespread adoption of cage-free systems, as seen in other mature markets such as Europe and the United States.

As we move forward, it is imperative for stakeholders in the egg production sector, including producers, policymakers, and consumers, to collaborate in fostering an environment where ethical and sustainable practices are not only encouraged but are also economically viable. The evidence presented in this document suggests that cage-free production holds promise not only in meeting the growing demand for ethically produced eggs but also in shaping a more compassionate and resilient food production system.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

JR: Writing – review & editing, Writing – original draft, Investigation, Conceptualization. AC: Writing – original draft, Investigation, Data curation. NS: Writing – review & editing, Validation, Supervision, Project administration. PW: Writing – review & editing, Validation, Project administration, Methodology, Data curation. MM: Validation, Writing – review & editing, Methodology. DB: Data curation, Writing – review & editing. AT: Writing – review & editing, Visualization, Resources, Funding acquisition.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Veterinary Science

TYPE Original Research
PUBLISHED 19 August 2024
DOI 10.3389/fvets.2024.1448232

OPEN ACCESS

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RECEIVED 13 June 2024
ACCEPTED 22 July 2024
PUBLISHED 19 August 2024

CITATION
Fox D, Wilson B and Narayan E (2024) Non-invasive wool hormone assessment of Australian merino rams (*Ovis aries*): a pilot investigation of cortisol and testosterone. *Front. Vet. Sci.* 11:1448232. doi: 10.3389/fvets.2024.1448232

Non-invasive wool hormone assessment of Australian merino rams (*Ovis aries*): a pilot investigation of cortisol and testosterone

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Introduction: Non-invasive hormone assessment is growing in interest as producers and livestock researchers seek new methods to assess animal welfare. Non-invasive wool assessment offers long-term, historic reflections of hormone concentration at the scale of weeks and months - and are not limited by sampling stress - thus making wool an appropriate tissue for long-term hormone analysis. This pilot study quantified cortisol and testosterone concentrations of ram fleece and determined if there is a significant difference between segments of the sample staple, and whether there is a correlation between hormones. Cortisol is a glucocorticoid produced within the adrenal glands and secreted in anticipation of or in response to a stressor. Testosterone is an androgen mainly synthesised within the testes of males and responsible for several critical functions including regulation of muscle growth, libido and spermatogenesis.

Methods: In our study, 70 topknot wool samples were collected from rams on a commercial stud property in Dirranbandi, Queensland, Australia. Of these animals, 12 samples were selected at random to undergo cortisol and testosterone quantification. In the laboratory, a single, intact staple was isolated from the total sample, divided into 10 mm segments and prepared for their respective (cortisol or testosterone) immunoassays.

Results: No significant difference ($p > 0.05$) was found between wool segments for either cortisol or testosterone, however, statistical differences ($p < 0.05$) were found between individuals for both hormones. A strong positive correlation ($R^2 = 0.9173$, $p < 0.05$) was found between wool cortisol and testosterone concentrations.

Discussion: In summary, this study reveals the major future possibilities for non-invasive wool hormone assessment in merino rams.

KEYWORDS

stress, non-invasive biomarkers, HPA axis, fibre, reproduction

Introduction

The stress response is a natural reaction to the perception of a threat and initially attempts to maintain homeostasis despite the stressor (1, 2). Stress can have significant effects on livestock (3). Cortisol is a steroid hormone in the class of glucocorticoids, colloquially

referred to as the 'stress hormone' for its role in the physiological stress response. Cortisol analysis provides an indication of activation of the hypothalamo–pituitary–adrenal (HPA) axis, which mediates the stress response, with support from the sympatho–adrenal–medullary (SAM) axis (4). Cortisol actively circulates the body via blood and is found in numerous bodily fluids, including saliva, excreta (urine and faeces), hair, and wool (5).

Testosterone, an androgen and the primary male sex hormone, is essential for the normal maturation of male animals because it promotes several critical developments, including protein synthesis, which contributes to the greater size of males, increased bone density, and proper penis, scrotal, and testes development (6). It is also necessary for spermiogenesis in male animals. In seasonal breeders, including the ram, reproductive axis activation occurs in relation to day length (7). Declining daylight stimulates oestrus behavior in ewes and elevated testosterone production in males (8). Testosterone is fundamentally important for libido and continual sexual activity by rams. Early research on growth rate and feed utilisation efficiency found that ram lambs outperformed their castrated counterparts and attributed the difference to testosterone (9–11) recognised that sexual activity increases with increase in testosterone levels.

Wool hormone analysis is a non-invasive method useful for retrospective studies due to the delay in hormone incorporation into wool (12, 13). Hair or wool has been used as a better alternative to blood samples for long-term hormone assessment (4, 14–18). Circulatory steroids are gradually integrated into the hair shaft whilst it grows, and as such, analysing the hair or wool of the animal can give a long-term record of the changes in steroids, such as cortisol, in the animal (4, 15–19). Steroids incorporated in the hair/wool shaft are not affected by the daily fluctuations of the hormones and can store the steroid changes for weeks or even months, depending on the species (4, 15–19). A study by Nejad et al. (15) that used both blood and wool samples in order to measure cortisol in sheep under heat stress and water restrictions concluded that cortisol in wool was a more accurate method of measuring stress in the animals used (15, 17).

The study objective is to evaluate whether there is a statistically significant difference between wool cortisol and testosterone across the length of the staple. It is hypothesised that no statistically significant difference will be found within the staple for each hormone.

Methods

Ethics approval

Biological samples were obtained with the University of Queensland ethics approval (with protocol approval number 2021/AE000485).

Fieldwork

Animal husbandry

All 70 rams (2 years of age) used in this study were owned by Wilgunya Merino Stud, located in Dirranbandi, Queensland, 4,486 (−28.856123236319117, 148.4624339024027). At the time of our visit (14 October 2022), all rams were housed in a separate paddock with *ad libitum* access to natural grasslands, shade trees, and fresh drinking water. Property managers used all terrain vehicles (ATVs) and sheep dogs to herd the rams into the race for sample collection.

Wool collection

Rams were run through the race in batches of 20 rams, where an identifying tag number was noted on both a resealable bag and a strip of paper, which was placed within the bag. Handheld electric clippers were used to recover a sample of topknot fleece (all rams were sampled at the same site on the mid forehead portion on top of the crown), which was placed within its own labelled bag. Rams were restricted in the race for an average of 10 min between sampling of the first and last ram. A total of 70 samples of ram fleece were collected. A wool sample was placed in a brown paper bag (labelled with ram ID and date) and kept in a plastic container at room temperature. All samples were collected by the farm support worker to reduce the risk of animal injury by inexperienced personnel. Upon return to the University of Queensland, Gatton Campus, 4,343 (−27.55331668592056, 152.3344294537852), all ram fleece samples were placed into a large vacuum-sealable bag and placed within a Waeco freezer at −20°C.

Laboratory analysis

Wool preparation

The methodology used was adapted from the study by Sawyer et al. (19). Before use, the vacuum-sealed bag was reinflated and allowed to reach room temperature before samples were catalogued. Of the 70 samples, a random selection (by handpicking) of 12 rams was made for this study. The main reason was due to the limited resources available to process and analyse all 70 samples (This could provide over 500 sub-samples in total). From each of these 12 ram wool samples, an intact, clean, unstretched staple was identified and gently removed from the fleece by hand. This piece was deemed the representative sub-sample for that individual moving forward. All other samples were returned to the freezer.

Using a ruler marked with 1 mm increments, each sub-sample was cut into 10-mm pieces [representing monthly wool growth in sheep; (12)] and assigned to a labelled weighing boat. The samples were labelled as "animal ID number" and a letter, ranging from A to G. For instance, 4C represented the third piece from ram #4. Sample "A" was furthest to the scalp, and "G" was the closest. The number of 10-mm sub-samples varied between rams due to differences in total staple length. The weight of all samples—minus the weighing boat weight—was recorded using a digital balance (Ohaus Pioneer™) accurate to three decimal places. To avoid human error, the balance was connected to a laptop via an RS-232 cable to allow for the direct transmission of weight data into Microsoft Excel.

Washing procedure

Early findings by Davenport et al. (4) using hair and subsequent studies using wool recommended the use of isopropanol as a washing agent to remove external contamination from the sample surface (19). As such, each 10-mm sample taken from the 12 wool sub-samples was submerged in 3 mL of 100% isopropanol for 5 min, drained, and placed within a glass desiccator until dry. Each 10-mm sample was typically dried within 48 h of washing and was then diced finely using

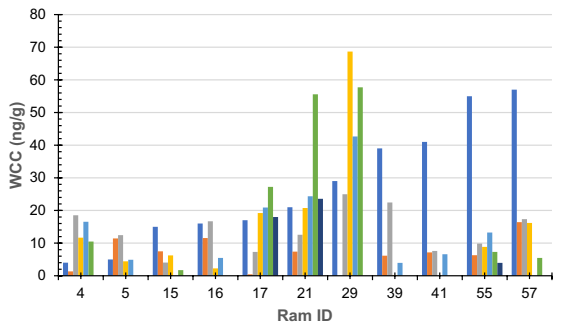

FIGURE 1
Wool cortisol concentration by 10-mm segment of sub-sample wool staple taken from rams (n = 11). Colours denote each segment of sub-sample wool (dark blue-a; orange-b; grey-c; yellow-d; light blue-e; green-f; and dark green-g). Ram sub-samples or 10-mm sections were as follows: Ram#4 = 5, Ram#5 = 4; Ram#15 = 4; Ram#16 = 4; Ram#17 = 6; Ram#21 = 6; Ram#29 = 4; Ram#39 = 4; Ram#41 = 3; Ram#55 = 6; Ram#57 = 4; and Ram#70 was removed due to contamination.

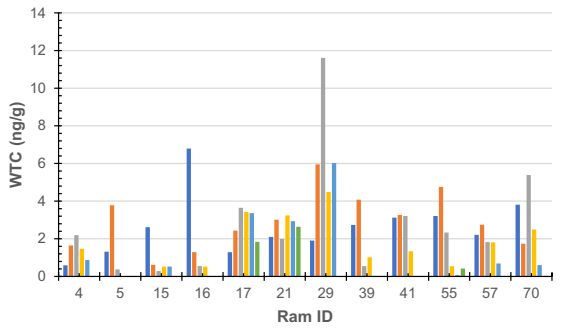

FIGURE 2
Wool testosterone concentration by 10-mm segment of sub-sample wool staple taken from rams (n = 12). Colours denote each segment of sub-sample wool (dark blue-a; orange-b; grey-c; yellow-d; light blue-e; green-f; and dark green-g). Ram sub-samples or 10-mm sections were as follows: Ram#4 = 5, Ram#5 = 3; Ram#15 = 5; Ram#16 = 4; Ram#17 = 6; Ram#21 = 6; Ram#29 = 5; Ram#39 = 4; Ram#41 = 4; Ram#55 = 6; Ram#57 = 5; and Ram#70 = 5.

forceps and surgical scissors and added to an Eppendorf tube with 1 mL of 100% ethanol for hormone extraction. The Eppendorf tubes were briefly vortexed for at least 1 s to ensure maximum ethanol penetration of the finely cut wool. The tubes were labelled and placed in a refrigerator.

Cortisol assay

Cortisol concentration was determined for 11 rams with each 10-mm sample that was obtained and extracted (a total of 50 wool sub-samples had testosterone measured; and the number of sub-samples per ram is shown in the legend of Figure 1) using the DetectX® Cortisol Immunoassay kit, manufactured by Arbor Assays. Briefly, a pipette was used to extract 500 µL of each sample into new, labelled Eppendorf tubes. The tubes were placed in a fume hood with the caps open to dry overnight. The remaining methanol sample was kept in the refrigerator for any future work. After drying, the samples were reconstituted with 25 µL of 100% methanol and 475 µL of ELISA assay buffer, which was prepared by diluting the assay buffer concentration provided in the DetectX® Cortisol Immunoassay kit with distilled water in a ratio of 1:5.

Nunc™ 96-well plates were coated with 50 µL of cortisol antibody solution and incubated for at least 12 h. Standards were prepared serially using 200 µL of standard stock and 200 µL of assay buffer. Cortisol standards were run in duplicate from 400, 200, 100, 50, 25, 12.5, 6.25, 3.12, and 1.56 pg./well. Four non-specific binding (NSB) wells were used and included 75 µL of assay buffer was used and 50 µL of assay buffer to the two maximum binding wells (labelled "0" on the plate map. A volume of 25 µL of DetectX® Cortisol conjugate was added to every well along with 25 µL of DetectX® Cortisol antibody, which was omitted from the NSB wells. Once loaded, the plate was covered using sealing film, labelled with the assay type and time, and set atop the plate shaker at 900 rpm for 1 h. Next, the plate was washed four times using an automatic plate washer. Once washed, the plate was gently tapped dry using a paper towel. After adding 100 µL of tetramethylbenzidine (TMB substrate) to each well, the plate was resealed and allowed to incubate at room temperature for 30 min. Then, 50 µL of stop solution was added to each well. The plate was read at 450 nm using a microplate reader. Cortisol concentrations were exported as a CSV. Data were transformed from absorbance values (AU cm-1) into cortisol concentrations provided in nanograms per gram (ng/g).

Testosterone assay

Testosterone concentration was determined in 12 rams (a total of 53 wool sub-samples had testosterone measured as indicated in Figure 2) using the R156/7 enzyme within an immunoassay. A 96-well Nunc™ Maxisorp plate was coated the day prior to the assay. A volume of 25 µL of antibody stock was added to 6,225 µL of coating buffer at a working dilution of 1:25,000. The first column was reserved as non-specific binding wells and was coated with a coating buffer and without an antibody. Then, 50 µL of antibody was added per well using a multi-pipette. The plate was gently tapped on the table to maximise antibody coverage, covered and left for at least 12 h at 4°C.

Standards, including zeros and NSB, were prepared the following morning. Standard values were run in duplicate from 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, and 0.39 pg./well. The standard working stock was diluted serially two-fold using 200 µL of stock and 200 µL of EIA buffer. This was repeated for all the remaining standards. The samples were diluted in EIA buffer. Then, 15 µL of testosterone conjugate [horseradish peroxidase (HRP)] was added to 5,985 µL of EIA buffer to create the working dilution.

An automatic plate washer was used to wash the plate four times with a wash solution. A paper towel was used to gently dry any remaining wash solution from the plate after washing. Using the plate map as a guide, 50 µL of standard, control, and sample were each added per well. Then, 50 µL of diluted testosterone HRP was added to all wells that contained standard, control, or sample. Within 10 min of beginning, the loaded plate was covered and left to incubate at room temperature for 2 h. Immediately after this time, the plate was washed four times with a wash solution using the automatic plate washer. The plate was briefly inverted to remove excess solution and carefully dried using a paper towel.

A volume of 50 µL of TMB substrate was added to wells that contained standard, control, or sample. The plate was covered with

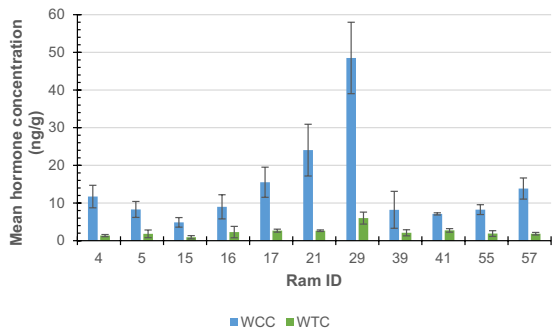

FIGURE 3
Ram wool mean cortisol (WCC; blue) and testosterone (WTC; green) concentrations.

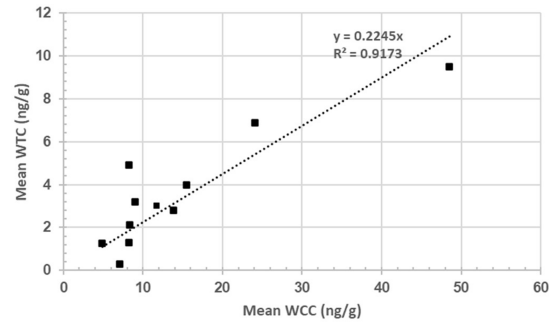

FIGURE 4
Relationship between wool cortisol concentration (WCC; ng/g) and wool testosterone concentration (WTC; ng/g).

adhesive film and left to shake for 30 min for maximum colour development. A measure of 50 µL of stop solution was added to wells that contained standard, control, or sample. The plate was inserted into the plate reader and read at 450 nm. Absorbance values were exported as a CSV, imported into Microsoft Excel, and transformed to testosterone concentration (ng/g).

Statistical analysis

Cortisol and testosterone absorbance data (AU cm^{-1}) were provided by the plate reader as a CSV, which was transformed into both cortisol and testosterone concentration (ng/g) in Microsoft Excel. The first analysis included descriptive statistics, including mean, standard deviation, and standard error. A few outlier values were found. All such values were removed from all calculations. This included samples (10-mm sections) for cortisol analysis in 5E, 15D, 41C, 57D, 29A, and 70D due to random error. The samples (10-mm sections) removed from testosterone analysis included 5E and 5D due to random error. Wool hormone levels were compared within sub-samples and between individuals using a one-way ANOVA. All hormone data were log-transformed prior to analysis. Regression was performed to determine the relationship between wool testosterone and wool cortisol. A p-value of <0.05 represented levels of significance.

Results

Wool cortisol

Raw WCC for each ram is presented in Figure 1. No statistically significant ($F = 0.93$, df = 5, 44, $p = 0.46$) difference was found between an individual ram's sub-sample; however, a statistically significant ($F = 2.27$, df = 10, 39, $p = 0.03$) difference was found between individual rams.

Wool testosterone

No statistically significant ($F = 1.33$, df = 5, 52, $p = 0.26$) difference was found between an individual's samples; however, a statistically significant ($F = 2.17$, df = 11, 46, $p = 0.03$) difference was found between individuals. Raw WTC for each sheep is presented in Figure 2.

Wool cortisol and testosterone

As shown in Figure 3, the X-axis represents the ram with both cortisol and testosterone results ($n = 11$; notably, ram #70 was removed from cortisol analysis) identification number and the y-axis represents the hormone concentration, measured in ng/g. Error bars depict the standard error (SE) of each data set, which measures variability around the mean.

The scatter plot represents the mean WCC ($n = 11$) and mean WTC ($n = 11$) for rams #4, 5, 15, 16, 17, 21, 29, 39, 41, 55, and 57. The linear regression intercept line is determined by the equation y = 0.2245x, suggesting WTC increases by 0.2245 ng/g per ng/g of WTC on average. The R^2 of 0.9173 suggests a very strong, positive correlation ($p = 3.65995E-05$) between both hormones (Figure 4).

Discussion

Wool hormone assessment

This study assessed the variation in wool cortisol concentration (WCC) and wool testosterone concentration (WTC) along the length of the staple. No statistical difference ($p > 0.05$) in either wool cortisol or testosterone was found along the length of the staple. These findings are consistent with the study by Hantzopoulou et al. (20), who reported no statistically significant difference in the wool cortisol concentration when sub-sampling Merino ewe topknot fleece. Similarly, Davenport et al. (4) found no significant difference ($p > 0.05$) in cortisol between the distal and proximal portions of monkey hair. These results are replicated in a human hair study by Yang et al. (21), who found no significant difference in hair cortisol at three different lengths. Caution, however, must be applied during inter-species comparisons as several biological differences are at play. For instance, the use of human hair products is known to alter the hormone profile of hair. Nonetheless, it demonstrates that—to date—the assessment of hair segments is yet to yield a statistically significant difference, at least under mild stressors.

The present study did find a statistically significant difference ($p < 0.05$) in WCC and WTC between individual rams, demonstrating that the hormonal activity of individual rams varies within the flock. Hormones are chemical secretions that facilitate communication

between bodily systems. Cortisol is the primary hormone of the mammalian stress response and is released by the adrenal glands, where it enters the bloodstream. Although the exact mechanism of incorporation is disputed, strong evidence supports the passive diffusion of hormones into the hair shaft through blood (1). As such, wool hormone concentration varies from animal to animal as a direct reflection of the level of HPA axis activity.

Our hypothesis, which suggested that there is no statistically significant difference in both wool cortisol and testosterone along the length of the staple, was proven correct. Additionally, we also confirmed that wool hormone concentrations vary significantly between individuals. As the rams were cohabitating under similar environmental conditions and much effort was taken to eliminate potential stressors, it is reasonable to expect similar hormone results, as each animal was experiencing comparable levels of HPA axis activity.

Wool cortisol and testosterone relationship

Within many mammalian animal breeding populations, males actively compete against rival males in a dominance hierarchy for access to females. It is often the case that testosterone levels are related to the number of dominance disputes they have won and thus their position in the dominance hierarchy (22). Cortisol levels, however, vary across species, with examples of dominant males exhibiting low and higher blood glucocorticoid concentrations.

A positive association ($R^2 = 0.91$) was found between WCC and WTC, which appears to be the first documented case of such a relationship in rams (Figure 4). A study by Medill et al. (22) found a non-significant, positive association ($p > 0.05$; $R^2 = 0.43$) between hair cortisol and testosterone in feral horses. Bachelors (males of breeding age yet to win access to their own mare) were hypothesised to have lost more dominance disputes and thus have elevated cortisol levels (22). Although such a strong association is interesting—particularly in social animals, such as rams—it is difficult to confidently identify the exact reasoning for such a relationship from a single wool sample. Moreover, as wool samples were collected in October 2022, it is highly likely that seasonal changes that accompany the breeding season (February to June) are reflected in the samples and contribute to intra-sample hormone variability.

Future iterations of this study should first determine if seasonal hormone variability is reflected in wool, as in conventional blood samples. Second, in-field observations should be conducted to identify dominant rams within the flock, and later, repeat sampling and data analysis should be conducted to determine how wool cortisol and testosterone change alongside shifts in ram dominance hierarchy positioning.

Conclusion

Wool could provide a suitable, non-invasive biological matrix for the assessment of ram cortisol and testosterone, avoiding the need for blood collection or faecal sampling. No statistically significant differences were found along the length of the staple for either hormone. These findings were confirmed by the literature. As hypothesised, a statistically significant difference was found between individuals. Measuring wool cortisol and testosterone provides an indication of the activity of the HPA and male HPG axes. Moreover, a strong correlation was found between wool cortisol and testosterone concentrations. After an extensive literature search, it is believed this study is the first to find such an association between reproductive and stress hormones in wool.

Data availability statement

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was approved by Animal Ethics Committee—The University of Queensland. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

DF: Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. BW: Resources, Writing – original draft, Writing – review & editing. EN: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. We would like to acknowledge the contributions made by the Australian Wool Education Trust (AWET) for the scholarship and partial funding for this project.

Acknowledgments

This research was conducted as a part of a Master of Animal Science Research thesis for DF and supervised by EN during the manuscript draft stages. Members of the Stress Lab including Harsh Pahuja and Georgia-Constantina Hanztopoulou provided support with lab work. We would like to acknowledge Benn and Max Wilson of Wilgunya Merino Stud for the collaboration.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

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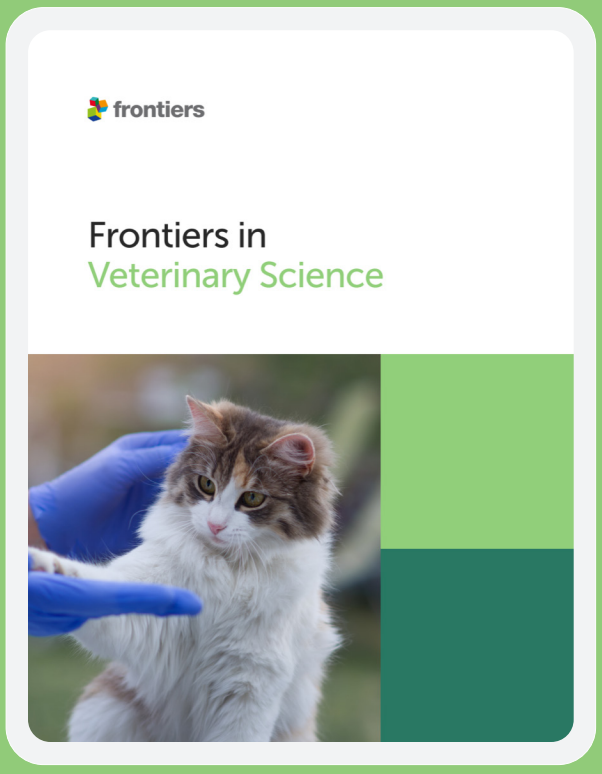

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