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The emergence of animal welfare science and policy in Africa, Asia and Latin America

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The emergence of animal welfare science and policy in Africa, Asia and Latin America

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Editorial: The emergence of animal welfare science and policy in Africa, Asia and Latin America

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Editorial on the Research Topic
The emergence of animal welfare science and policy in Africa, Asia and Latin America

As part of efforts to raise the profile of animal welfare science within Africa, Asia and Latin America, this Research Topic was generously supported with full article processing charge remission by Frontiers Media SA, to enable scientists to publish their work in a high-quality Open Access journal. For many countries within these regions, animal welfare science is still nascent and this Research Topic highlights some of the animal welfare issues within these regions and the local scientific research being directed to find solutions. The result is a diverse collection of papers covering farm, laboratory and zoo animals.

Animal welfare science as a discipline, has a relatively modern history. Although good treatment of animals is an important tenet of some religions and civilizations dating back a few millennia, for example the concept of Ahimsa in Jainism, Buddhism, Hinduism, and Sikhism (1), the formation of policy and enactment of legislation has almost exclusively been a 20th Century and onwards phenomenon. Although the first known animal protection legislations were passed in Ireland and the Massachusetts Colony in 1635 and 1641, respectively (2), and anti-cruelty legislation for cattle and other animals passed in the U.K. in 1822 and 1876, the catalyst for more widespread welfare-focused legislation and for the emergence of animal welfare science was Ruth Harrison's book Animal Machines (3) and the subsequent Brambell Report established by the UK Government (4).

Within the Brambell Report was the embryonic text of what evolved into the Five Freedoms, and also Appendix III (5) which detailed the scientific assessment of pain and distress in the principal farm animal species. From these acorns, animal welfare as a scientific specialty grew, though not without growing pains and indeed still some suspicion from some veterinarians and animal scientists in particular. With the entry of the UK into the European Union in 1973, animal welfare became an EU-level issue (6), with formation and expansion of funding for animal welfare science, and formation of advisory bodies, ultimately the

European Food Safety Authority, to collate, interpret and report on the science of animal welfare in order inform policy and legislation. With the appointment of Prof. Donald Broom to the world's first Chair in Animal Welfare in 1986 at the University of Cambridge, animal welfare science began its introduction into veterinary teaching, spreading across Europe and gradually further afield across the rest of the world. The World Organization for Animal Health began incorporating animal welfare into the Terrestrial Animal Health Code (7) in 2004, meaning 182 member countries across the world have approved the concept of animal welfare and the development and implementation of animal welfare standards. It is clear that animal welfare, and laws to protect animals, are important across the world (8).

However, it has been suggested that the historical spread of "Western" farming methods represented animal colonialism, defined as "a dual phenomenon, consisting, on the one hand, in using animals to colonize lands, native animals, and people and, on the other hand, in imposing foreign legal norms and practices of human-animal relations upon communities and their environments" (9). Therefore, as animal welfare science expands globally, we must be cautious that it retains its relevance to cultural issues, and that a "euro-centric" focus of animal welfare defined by its evolutionary origin is not imposed upon other cultures, in a form of neocolonialism (10). The answers to animal welfare issues within Africa, Asia and Latin America lie within these areas. Although we continue to see the spread of intensive farming systems and other animal uses into these regions (11), the animal welfare issues may be familiar ones, but may also be different. It is imperative that internal and external stakeholders invest in animal welfare science inside these geographic areas, both in terms of people—animal welfare scientists, lecturers, auditors, etc.—and infrastructure, and that local and national animal welfare issues are primarily addressed by local and national expertise.

A cursory Web of Science Core Collection search of the term "animal welfare" yields just under 25,000 papers. Of these, around 1,800 have authors based Latin America, 1,400 have authors based in Asia (excluding Japan) and 500 have authors based in Africa, illustrating the relative strengths of animal welfare science in the regions. This may also be reflected by the degree of collaboration with coauthors from outside the region. Although collaboration with scientists external to the region could have benefits in terms of English language publishing (Gallo et al.) and reducing conscious and unconscious biases in the publishing process, reduced collaboration can also indicate that animal welfare science is more established and that there is less need to collaborate. About 65% of papers from Latin America and Asia have within-region coauthors only but this drops to 40% for papers from Africa. For papers specifically addressing animal welfare within these regions, under 10% of papers concerning Latin America and Asia have no authors from those regions, but this increases to nearly 25% for animal welfare within Africa—i.e., a quarter have no local expertise input.

There is ongoing intensification of animal agriculture within all regions of the Research Topic (12), and the introduction of highly-selected breeds. This may result in a potential loss of indigenous breeds which are not only the mainstay of small-scale production—providing income and nutrition—but are also a valuable genetic resource (13). A better understanding of their behavior and welfare can impact survivability and efficiency of production, with corresponding human benefits, thereby safeguarding their preservation. A pair of papers on Nigerian indigenous chickens investigated differences in maternal care of hens and fear responses of chicks of two ecotypes (Oyeniran et al.) and the hens' responses to visual or physical separation from their chicks (Iyasere et al.). Both of these papers help to identify behavioral traits that might improve survival within the extensive systems in which the chickens are kept, with frequent exposure to predation. Also, increasingly important within Africa is aquaculture, with the two dominant species being tilapia and African catfish. Ojelade et al. investigated the impacts of providing environmental enrichment to catfish under laboratory conditions, and found advantages in growth rates and reduced aggression, warranting further research to determine potential application to commercial fisheries.

Animal welfare can only be improved with knowledge. This includes knowledge of the current status of the animal's welfare, knowledge of people's current perceptions of, and attitudes toward animal welfare (8), and knowledge about barriers that may be preventing adoption of ideas or mechanisms that may improve welfare, specific to the culture in which improvement is trying to be enacted (14). Assessment of current welfare is a good starting point from which to enact change. Romero et al. used a previously standardized and validated protocol with animal-based measures of behavior and health to assess welfare of horses and mules in Colombia by direct observation. Racciatti et al. developed a welfare assessment protocol including animal-, resource- and management-based measures that could be used across multiple zoo animal species, including mammals, birds and reptiles, again by direct observation. Resasco and Diaz surveyed laboratory mice breeding facilities in Argentina, using animal-, resource- and management-based measures to provide the first knowledge about welfare within such facilities. These assessments yield important information that can then be used to highlight areas of concern, develop training to address identified issues and inform future direction.

Lemma et al. explored animal welfare perceptions in rural households in Ethiopia using a Community Conversations methodology, using facilitated group discussions to identify community strengths and constraints, values and practices and explore strategies to address livestock management challenges. A survey of Ethiopian livestock-owning households is reported in Alemayehu et al., using a survey tool designed to measure participants' Knowledge, Attitude and Practice (KAP) of animal welfare. Community Conversations raise awareness and can serve as an effective way to channel community feedback into welfare improvement programs. The KAP methodology can help identify areas requiring targeted training. A survey of egg producers from 6 Asian countries (de Luna et al.) explored the benefits and challenges to adopting cage-free systems, showing that there is a widespread perception that caged systems have cost and ease of management advantages, but that cage-free systems are perceived as higher welfare. Nearly three-quarters of producers said more support is needed to establish cage-free farms, with technical advice, training and resources needed.

Another area of animal welfare that has received increasing scrutiny over the last few years is that of animal tourism. In some developing economies, these activities can be seen as important drivers of income into the country in general, as well as obviously directly impacting individual livelihoods. The COVID-19 pandemic brought travel and tourism to a halt, impacting human and animal welfare. Supanta et al. examined the impacts of COVID-19 on elephant camp management in Thailand, and the reduction in income lead to unemployment of carers, which itself could impact elephant welfare, and increased time spent chained and decreased nutrition.

Finally, the trends in farm animal welfare publications in Latin America were examined by Gallo et al.. Over the last 30 years, nearly 700 papers were identified on farm animal welfare produced by researchers in Latin American countries. However, 95% were published in the last 15 years, showing a rapid increase during this time, both in research and in training. Nearly 70% were produced by Brazilian and Mexican researchers and over 40% were on cattle, illustrating the importance of these countries cattle industries.

Overall, the quantity and quality of research being carried out in Africa, Asia and Latin America is increasing. The notion that animal welfare is important to more developed countries alone is false (8), and we must regard animal welfare as a key factor within sustainability and development frameworks (15), as we seek to improve the lives of all human and non-human inhabitants of our planet.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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.

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References

1. Kumar S. Veganism, hinduism, and jainism in india: a geo-cultural enquiry. In: L. Wright, editor. *The Routledge Handbook of Vegan Studies*. London: Routledge (2021).

2. Lane BC. *Ravished by Beauty: The Surprising Legacy of Reformed Spirituality*. New York: Oxford University Press (2011).

3. Harrison, R. *Animal Machines*. London: Vincent Stuart Ltd. (1964).

4. Brambell, FR. *The Report of the Technical Committee to Enquire into the Welfare of Animals Kept under Intensive Livestock Husbandry Systems*. London: HMSO command report no. 2836 (1965).

5. Thorpe WH. The assessment of pain and distress in animals. Appendix III in *The Report of the Technical Committee to Enquire into the Welfare of Animals Kept under Intensive Livestock Husbandry Systems*. London: HMSO command report no. 2836 (1965).

6. Simonin D, Gavinelli A. The European Union legislation on animal welfare: State of play, enforcement and future activities. In: Hild S, Schweitzer L, editors. *Animal Welfare: From Science to Law*. Paris: La Fondation Droit Animal (2019). p. 59–70.

7. World Organization for Animal Health. *Terrestrial Code. 7, Animal Welfare. Ed*. Paris: OIE (2022). Available online at: https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/?id=169&L=1&htmfile=titre_1.7.htm (accessed January 31, 2023).

8. Sinclair M, Lee NYP, Hötzel MJ, de Luna MCT, Sharma A, Idris M, et al. International perceptions of animals and the importance of their welfare. *Front Anim Sci.* (2022) 3:960379. doi: 10.3389/fanim.2022.960379

9. Cohen, M. Animal colonialism: the case of milk. *AJIL Unbound.* (2017) 111:267–71. doi: 10.1017/aju.2017.66

10. Garcia A, McGlone JJ. Animal welfare and the acknowledgment of cultural differences. *Animals.* (2022) 12:474. doi: 10.3390/ani12040474

11. Pandey HO, Upadhyay D. Global livestock production systems: classification, status, and future trends. In: S. Mondal, R. Lakhan Singh, editors. *Emerging Issues in Climate Smart Livestock Production*. Cambridge, MA: Academic Press (2022). p. 47–70.

12. von Keyserlingk MAG, Hötzel MJ. The ticking clock: addressing farm animal welfare in emerging countries. *J Agric Environ Ethics.* (2015) 28:179–95. doi: 10.1007/s10806-014-9518-7

13. Belew AK, Tesfaye K, Belay G, Assefa G. The state of conservation of animal genetic resources in developing countries: a review. *Int J Pharma Med Biol Sci.* (2016) 5:58–66. doi: 10.18178/ijpmbs.5.1.58-66

14. Sinclair M, Lee HP, Chen M, Li X, Mi J, Chen S Marchant JN. Opportunities for the progression of farm animal welfare in China. *Front Anim Sci.* (2022) 3:893772. doi: 10.3389/fanim.2022.893772

15. Keeling L, Tunón H, Olmos Antillón G, Berg C, Jones M, Stuardo L, et al. Animal welfare and the United Nations sustainable development goals. *Front Vet Sci.* (2019) 6:336. doi: 10.3389/fvets.2019.00336

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Using Community Conversations to explore animal welfare perceptions and practices of rural households in Ethiopia

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There is a scarcity of data on animal welfare and its impact on livelihoods to inform animal welfare initiatives in Ethiopia. Perceptions and practices of rural households toward animal welfare are influenced by socio-cultural, demographic, and agroecological factors. We conducted Community Conversations in two geographically and culturally diverse regions of Ethiopia to explore the attitudes and practices of rural households regarding animal welfare and its impact on livelihoods. Community Conversations are facilitated dialogues among rural households to explore their perceptions, practices, constraints, and needs and identify and co-create solutions to improve the welfare of their animals. We used single- and mixed-sex discussion groups to understand community members' gendered perceptions of animal welfare and influence their attitudes and practices toward gender-equitable roles in animal welfare management. In the Community Conversations, community members readily described the biological needs of their animals but there was also a good acknowledgment of the behavioral and affective state needs of animals. Identified constraints for animal welfare included feed and water shortage, limited veterinary support, and poor animal handling practices. Community members described the welfare of their animals as being intertwined with their own livelihoods and identified productive, public health, and non-economic benefits of good animal welfare. Raising awareness of animal welfare within rural communities through Community Conversations is a useful way to both identify livestock production needs as well as engage community members in making practical improvements in animal welfare. The understanding of perceptions, practices, and needs of rural households in animal welfare helps

engage communities in starting behavioral change and provides insights into developing context-specific welfare improvement interventions. Community Conversations are also an effective way to feedback community voices into planning to build a bottom-up implementation of animal welfare programs.

KEYWORDS

animal welfare, human-animal relationships, smallholder production, Community Conversations, Ethiopia

Introduction

The lives of animals and people are inextricably linked. Scientific research on animal welfare has predominantly concentrated on intensive production systems in the industrialized world (1). Research on animal welfare has been induced by public concerns over the welfare of animals kept in confinement production systems (2, 3). The concern about animal welfare has tended to emphasize different components of animal welfare. An integrated concept of animal welfare comprises the physical health and biological functioning of animals (such as freedom from disease, injury, and hunger), affective states of animals (like pain, distress, and pleasure), and the ability of animals to live reasonably natural lives by carrying out natural behavior and having natural elements in their environment (4).

The rising public and scientific concern regarding animal welfare has increased demands on governments and organizations worldwide to adopt animal welfare policies, legislations and regulations and create public awareness (5). More recently, there is also a growing body of literature focusing on public concerns and farmers' attitudes toward animal health and welfare (6).

While animal welfare has been a concern of developed countries for many decades, it has recently also gained more attention in low- and middle-income countries (LMICs) (7). It has become an important factor in trade in animal products and a concern for food safety and public health. For developing countries, like Ethiopia, to access global markets, it is crucial that international animal welfare and food safety standards are established and observed (8).

Animal welfare also contributes to the achievement of the United Nations Sustainable Development Goals (SDGs) and promotes the One Health approach. Caring for animals is a pathway to improving both human and animal wellbeing (9). Therefore, the disregard for animal welfare translates into negative impacts on human welfare as the welfare of human beings and animals is inextricably linked.

In Ethiopia, smallholder farmers and pastoralists play a key role in animal management and welfare (10). However, relatively little is known about animal health and welfare-related issues farmers and pastoralists find important and how that translates into good husbandry practices (11). There is a scarcity of data on how smallholder farmers and pastoralists perceive animal welfare, what their practices are, and their understanding of the relationship between animal welfare, productivity, and livelihoods (12). Previous animal welfare studies in Ethiopia have mainly focused on animal transport and slaughter (13), but little has been done at the level of farmers and pastoralists. While the big problems are occurring during transport, the problems should nevertheless not be ignored at the farm level where the animals spend most of their lives.

Using Community Conversations, this study contributes to the body of scientific knowledge on animal welfare by exploring the perceptions, constraints, needs, and practices of smallholder farmers and pastoralists in animal welfare in a developing country context. Community Conversations are powerful tools to engage community members in collaborative learning, reflection, and problem-solving, and facilitate community outreach through social learning and peer influence (14). The practical purpose of the study is to improve the welfare of animals and humans by changing the attitudes and practices of animal owners in developing countries.

Materials and methods

Description of the study sites

In October 2019, Community Conversations on animal welfare and livelihoods were conducted in two rural communities in Ethiopia: Darito community in Yabello district of Oromia region, and Sine Amba community in Menz Gera district of Amhara region. These were sites where the Consultative Group on International Agricultural Research Program on Livestock (CRP Livestock) implemented livestock research interventions to improve the livelihoods of smallholder livestock producers. The sites were selected based on their livestock density, agroecology, and agricultural production systems.

The study sites are linguistically, culturally, and agro-ecologically diverse. The population in the Menz Gera site dominantly follows Orthodox Christianity and belongs to

the Amhara ethnic group. The population in the Yabello district belongs to the Oromo ethnic group and the majority of the Borana people are Muslim although some practice traditional religion. The agroecology and production system characteristics of the study sites are shown in Table 1. Livestock production in Ethiopia is broadly classified into pastoral, agro-pastoral, and mixed crop-livestock production systems. With an altitude of 2,800–3,100 meters above sea level (masl), the topography of the Menz Gera district consists of plain, mountain, gorge, and undulated land features. The district has bimodal annual rainfall between 900 and 1,000 mm with a mean annual temperature of 12°C. The agricultural production system of the Menz Gera district is a highland mixed crop-livestock production system dominated by crops (15). Livestock production, especially cattle and small ruminants, remains the main source of livelihood for the population.

Yabello district is classified as arid and semi-arid rangelands, with pockets of sub-humid zones. The rangelands are dominated by savanna vegetation, with varying proportions of open grasslands consisting of perennial herbaceous and woody vegetation (16). The district has a pastoralism and agro-pastoral production system dominated by livestock production which remains the main source of food, income, and social prestige. Livestock husbandry in lowland agroecology is dominated by goats, cattle, sheep, and camels. With an altitude of 350–1,800 masl, the Yabello district has bimodal rainfall. The mean annual rainfall is 500 mm with considerable inter-annual variability and the mean annual temperature is 24°C (17).

In the study sites, livestock forms an important part of the livelihoods of the communities (10). Feed and water shortages and animal diseases are the major livestock production constraints (18, 19). Women and men play different roles in livestock management (20). Women are commonly involved in feeding animals, cleaning barns, caring for small and sick animals, and milking cows. Men are responsible for gathering or purchasing animal feed and herding and watering animals in distant locations. However, gender norms and practices as well as the weak gender capacity of service providers limit women's access to and use of livestock services including information and training (21).

The Community Conversation approach and process

Community Conversations are participatory engagement and learning processes where community members and local service providers work with trained facilitators to collectively identify community strengths and constraints, analyze community values and practices, and explore strategies for addressing livestock management challenges (22, 23). They encourage critical discussions and reflections among community members and local service providers on pertinent livestock development issues leading to the development of community actions to make desired improvements. The Community Conversations approach has its roots in participatory approaches such as social learning theory (24), actor-oriented approach (25), participatory learning and action (26), and participatory action research (27).

Drawing upon principles and practices of these participatory approaches, we designed Community Conversations protocol (28). The protocol provides methodological guidance and process steps for the implementation and documentation of Community Conversations. The approach has already been tested and documented in the CRP Livestock in Ethiopia addressing different livestock management issues (14, 23). It involves iterative learning, action and reflection steps: (1) exploration and analysis of existing community knowledge and practices; (2) introduction of new knowledge to address community knowledge and practice gaps; (3) learning integration and reinforcement through the communication of action messages; and 4) community actions and mentoring support (14). A range of active learning methods, including posters, pictures, storytelling, role-plays, provocative questioning, and personal reflections, were used in the Community Conversations. The use of illustrations such as posters and pictures encouraged the active participation of community members and provided a structure to guide the conversations.

We formed a team of local facilitators comprising research and development partners who have familiarity with the communities and speak the local languages. In the Yabello district, we worked through local translators. The local partners played key roles in contextualizing or localizing the discussion issues, facilitation, and documentation of the Community Conversations. We trained the local partners on the methodological approach, facilitation and note-taking protocols.

Based on developed criteria, together with the local partners, we selected 94 community members (42 women including married and household heads) and 16 (5 female) local service providers in the study sites. In identifying the participants, we strived for a diversity of opinions and perspectives to achieve a richer dialogue, collaborative learning, and community actions. We used single- and mixed-sex discussion groups to explore community members' gendered perceptions of animal welfare and influence their attitudes and practices toward gender-equitable animal welfare management.

The study was planned with local authorities, and they gave their approval for the work and played an active role in the implementation. Oral consent was obtained from the community participants prior to the commencement of the Community Conversations. Human ethics approval was obtained from the Institutional Research

TABLE 1 Description of the study sites.

Region	District	Community	Agroecology	Production system	Altitude (m)	Rainfall (mm)	Temperature (^{0}C)
Oromia	Yabello	Darito	Dry lowland	Mixed crop-livestock	1,800	500	24
Amhara	Menz Gera	Sine Amba	Moist highland	Mixed crop-livestock	3,100	900–1,000	12

Ethics Committee of the International Livestock Research Institute (ILRI-IREC2018-10).

Community Conversations discussion topics and questions

Engaging about 55 participants and running typically through 3–4 h in each study site, the Community Conversations explored the following open-ended discussion questions (Table 2). The topics and discussion questions were used as a checklist to guide the conversations and probing techniques were used to have a deeper understanding of the issues.

TABLE 2 Community Conversation topics and discussion questions.

Topics	Discussion questions
What is animal welfare? Why is it important?	• Can animals be happy or sad? Do they have feelings like humans? • How do you know when animals are sad or happy? • What makes animals happy or sad? Do you think animals suffer from physical pain? • Why is it important for animals to perform their natural behaviors? • What does animal welfare mean to you? What is the local term for animal welfare? • What are community members' attitudes and values toward animals? Are there any traditional customs, beliefs, or sayings about animals or their care? How are animals perceived or viewed in the community? • How do you describe good or bad animal welfare conditions in your community? Who in this community is regarded as the best animal caregiver? What makes this person the best animal caregiver? • When is moving or handling your animals easy? Does this differ by age, gender, personality, or experience of the handler? • What do you think are the effects of good and bad animal handling on the animal and the handler? • What do you think are the benefits of improving the welfare of your animals?
What are community members' animal welfare constraints, needs and options? How do these differ by gender?	• What do animals need to be healthy, happy, and productive? • What could happen if these animal needs were not met? • How do you observe or identify these effects on the animals? • What are the most common animal welfare issues that affect all species of animals in the community? • How well do you think you are meeting the needs of your animals? What are your constraints, needs, and options to improve the welfare of your animals? • What are the risks and opportunities for women and men in improving the welfare of their animals?

Data collection and analysis

We used process documentation to collect qualitative data on the Community Conversations implementation process and outcomes. Process note-taking tools and reflection checklists were used to record conversation results, reflect on the process, summarize emerging themes, interpret results, and draw lessons, which were documented in reflective reports (29). An after-event reflection and insight-making process with the facilitation team facilitated on-the-spot analysis, interpretation, and validation of Community Conversations results and experiences.

An inductive thematic analysis (30), which involves content analysis from documents, was used to analyze data contained in the research reports and field notes. We carefully reviewed the research reports and sought for thematic patterns to establish emerging themes and key findings and illustrate these with direct quotes from community members. The themes were also complemented with the literature to add context and validity. We considered socio-cultural, demographic, and agroecological factors in making a comparative data analysis.

Results

Multi-dimensional understanding of animal welfare

In the Community Conversations, community members demonstrated a basic understanding of animal welfare. Figure 1 illustrates the Community Conversations process and the main results. Community members stated that "animals have feelings like humans" and identified the conditions in which animals can be happy or sad and the behavioral responses of animals in those conditions. They said that animals are happy during

rainy seasons because they get enough feed and water. When there is rain, animals show signs of happiness like playing with each other and putting their tails up while running. They are sad during a drought season. Animals are unhappy when they are hungry, their shelter is unclean, they are sick, they get injured, or they are beaten. When they are unhappy, they have their head down, and they do not want to run and play. When they are not fed well, they do not want to go to their shelter; they want to go away, and they do not allow their offspring to suckle. A woman participant said, "animals are sad and feel bad when there is no feed and water, and when they are sick." Community members stated that animals can suffer from diseases or physical injuries when they do not receive good care or treatment.

In the study sites, community members recognized the need for their animals to express natural behaviors. They said that when animals are tethered or kept indoors all the time, it is not good for their health and body condition. For example, in Menz Gera, a woman participant said, "they become weak." Community members knew when their animals express the need for free movement. They said that animals show behaviors like making loud noises, becoming restless, and fighting one another. "When they are released from their shelter", a women farmer said, "animals run freely, and they love grazing in the open". Another woman said, "when animals get refreshed, they rest peacefully and longer in their shelters."

In Yabello, community members described animal welfare as "fulfilling what animals need and not adversely compromising their feelings." Similarly, in Menz Gera, community members described animal welfare as "*kibkabe*" meaning ensuring the wellbeing of animals or giving them good care. The community members described animal welfare to include having clean housing, timely feeding, leaving animals freely in the environment, not tying animals all the time, giving animals protection from predators, watering animals freely, and keeping them healthy.

In describing animal welfare, community members commonly associated feeding and health with the welfare of animals. They readily identified the biological needs of animals such as health, clean shelters, clean water, and sufficient feed. However, it was not obvious for them to identify the affective state and natural behavior of animals. These components of animal welfare did not come to their mind at first. It was through follow-up probing questions that they started to recognize these components of animal welfare.

Through the Community Conversations, community members described good and bad animal welfare conditions and assessed their own existing animal management practices. They said that the welfare of their animals is affected during drought due to a shortage of feed and water. They described bad animal welfare as keeping animals in dirty housing, withholding treatment, and disturbing animals by beating or yelling at them. The community members believed that animals need clean and comfortable shelter. They said that "animals refuse to enter unclean and wet shelters, and they rest for a shorter time in uncomfortable shelters." While recognizing good animal welfare conditions, community members also identified their limitations in giving good care to their animals, mainly related to resource constraints and handling behaviors.

Community attitudes and values toward animals

In the study sites, livestock is the main source of livelihood, social status, and prestige. Community members stated that they value their animals as they have no other options for living rather than their animals. In Yabello, women community members said, "our animals are many things for us. Cows give us milk, and bulls are used for plowing. Camels and donkeys are used for transportation. Sheep and goats are income sources to purchase household consumables."

In Menz Gera, community members said, "the existence of an animal is its owner" meaning it is the owner who provides care and protection to the animal. From cultural and religious perspectives, they argued that "it is a sin not to give care for animals", and they believed that "animal cruelty can cause judgment in heaven".

In Yabello, community members demonstrated closer connection and proximity with their animals. During the Community Conversations, they explained that they understand well their animals and express feelings about them, especially cattle. They stated that animals know their names and follow instructions from their owner or associated family member. Women and men community members used songs to communicate and connect with their animals. Women sang for their animals, especially dairy cows, a song called "*sirba*", welcoming them in the evening and facilitating milking. The song conveys the importance of animals as the source of subsistence for the household. It literally means that women give birth to children, and animals feed the children to grow. Similarly, male community members sang for their animals, especially cattle, a song called "*weedduu*" during plowing farm plots or when herding animals. The songs signify the importance of animals in the social status or prestige of male community members. In addition, the songs show the connections animal owners have with their animals. Expressing feelings about their animals using songs have a positive impact on the affective state of both the owners and the animals. The songs portray positive attitudes of animal owners to their animals, which is associated with more humane behavior toward the animals.

The songs also showed that women and men community members attached different values or meanings to animals depending on the purpose of the animals and their relationships

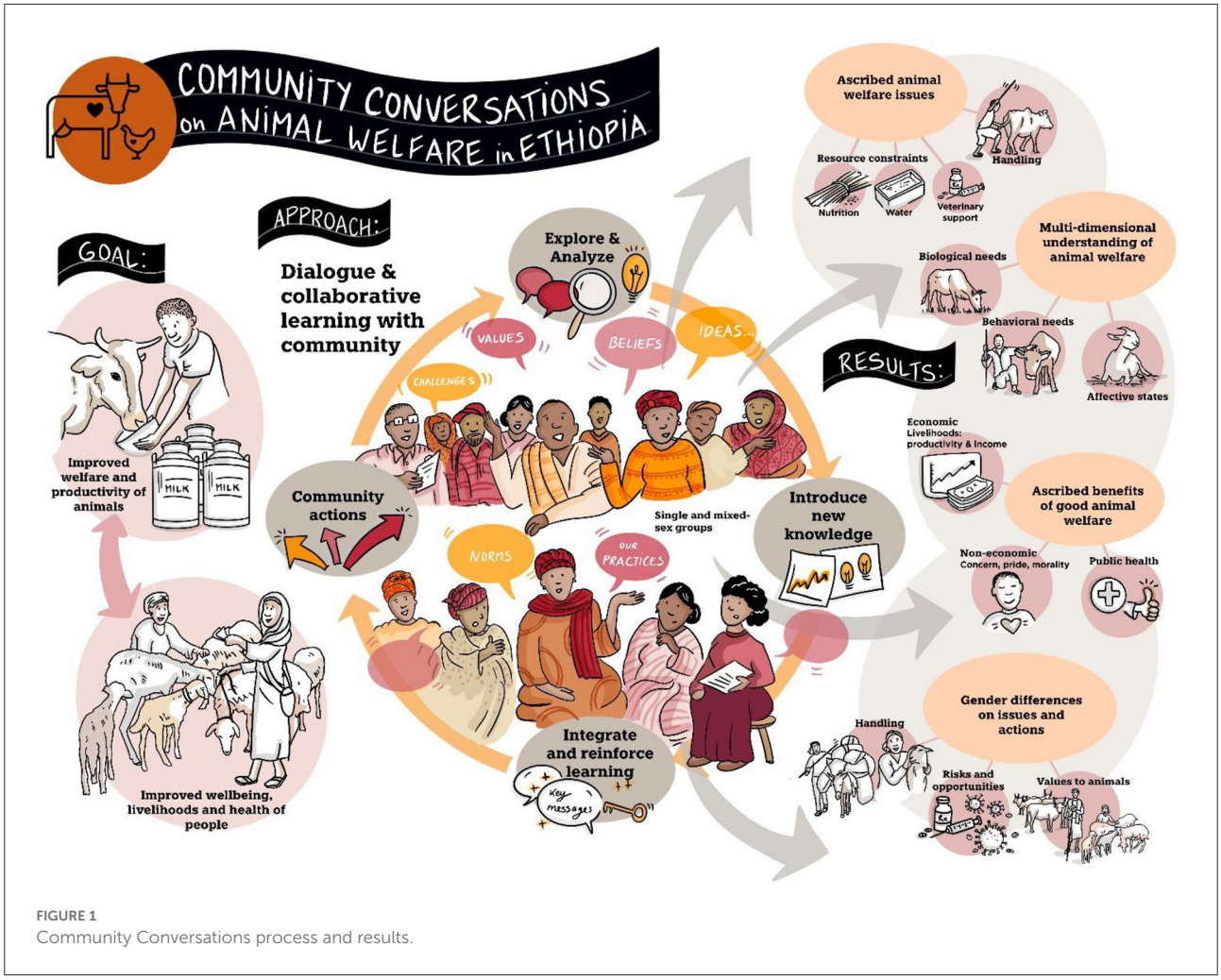

FIGURE 1
Community Conversations process and results.

with the animals. Community members gave more value and care to cattle, especially plowing oxen and milking cows, followed by small ruminants. As women focus on the provision of food and household wellbeing, they value and have a closer relationship with dairy cows, while men focus on social status or prestige, and thus attach more value to cattle. While pack animals such as donkeys play a key role in a rural economy, they received lower levels of welfare.

Although community members knew the value of animals, there was limited knowledge of what their animals needed to experience good welfare. When it comes to good animal management practice, there were limitations both due to resource constraints, lack of knowledge, and behavior of owners or caregivers. Their knowledge of diseases and the actual care they give to animals in terms of preventive measures was limited. There was also a knowledge gap regarding nutrition, behavioral and health problems of animals.

Community perceptions of human-animal relationships

While community members expressed positive attitudes and values toward animals, they also identified gaps in handling and giving good care to their animals. In Menz Gera, men and boys were reported to hit and yell at animals. A woman participant narrated that once her son tied up the legs of a sheep and beat it. Another woman said, "when I was driving my loaded donkey to a milling house, it refused to go. I requested a man to help me move the donkey. He beat it harshly, but the donkey did not move. Then he stopped beating the donkey and said he was sorry for my husband who has to handle the donkey".

On the contrary, in Menz Gera, women were reported to handle animals in a calm and friendly manner. Women participants explained that animals learn and develop fear if they are beaten or yelled at. A woman participant said, "animals run away, stop or change the direction of movement when they

hear the voice of children or male members of the household." Animals know who is taking good care of them, and they make noise when they see the caregiver or hear their voices. A male participant said, "animals behave like children. If I treat my son positively, he will call me father and approach me affectionately. Likewise, animals also know who gives them good care and express their connection with the handler."

Community members believed that animals should be handled properly. In Menz Gera, a male participant stated that "if we force and handle animals harshly, they will not move, they can be injured, or they could kick the handler." They explained that good handling is not only beneficial for the animals but also for the handler. When animals are handled badly, they can be aggressive, difficult to handle, and can injure the handler by kicking or biting. Bad handling of animals also affects the emotion of handlers. A male participant said, "I feel guilty when animals experience physical pain due to bad handling."

In Yabello, community members reported that they handled their animals calmly and never used force. They called their animals by name, restrained them by a rope, and showed them friendly behavior for easy handling. Community members reported that they never beat their animals harshly and do not yell at them. They indicated that they use different physical restraining techniques to manage fearful, strong, and aggressive animals.

In Menz Gera, although community members described donkeys as "beating tolerating animals", they believed that "all animals can feel physical pain as humans do and become unhappy or frustrated when they are harshly beaten." A woman participant said, "it is only stone that does not feel pain." Animals can become fearful and want to run away from humans when they are shouted at. "When animals experience physical pain or are worked hard", community members said, "they become fearful, have stripes on their skin when beaten, bend their bodies, fall on the ground, and do not move."

Male participants reported that ox beating during plowing was common, and if the ox were lazy, the beating was harsh. They even became aggressive when beating unresponsive animals. They said, "though we know that oxen feel physical pain, our focus is on finishing the plowing." A participant said, "it is the sunset which sets the oxen free", meaning the oxen are overworked throughout the day, especially during planting seasons. Another participant said, "a farmer who missed plowing in September cannot recover in September of the next year", meaning the month of September is a peak plowing season. After plowing, farmers said, "we massage the skin lesions or strips on the oxen and provide feed and water, but the oxen refuse to eat or drink, and this makes us feel bad."

While community members described the behavioral responses of animals due to negative handling, they were not aware of how negative handling can affect the health, growth, and productivity of their animals. Through the Community Conversations, community members recognized the effect of good and bad animal handling on the affective state, health, and productivity of their animals. They understood that animals need safety and relaxation in their handling and expressed commitment to handle their animals by gentle instruction rather than by beating the animals.

Ascribed benefits of good animal welfare

The Community Conversations showed that community members had a good understanding of the relationship between animal welfare and their livelihoods. Community members stated that their livelihoods depend on animals and the animals also depend on their owners. Describing this reciprocal effect, a male farmer said, "to benefit from animals, we have to take care of them. It is a give-and-take relationship". The community members also recognized the relationship between animal welfare and productivity. They stated that "when our animals are kept in good condition and are not stressed, they behave well and become productive. From our cows, we get good milk; healthy and strong bulls plow the land well". A woman participant said, "when milking I calmly handle my cow calling her by name and massaging her rather than beating or yelling at her. This way, my cow stops by herself for milking and gives more milk (does not withhold the milk). Also, when I keep her house clean, I get hygienic milk". A farmer in Menz Gera said, "keeping animals in good condition will save treatment costs".

Community members also realized the public health benefits of improving animal welfare. They argued that keeping their animals healthy and in good condition also means keeping their household members in good health and well-being. A woman participant in Menz Gera said, "if animal shelters are not clean and dry, a bad smell can cause respiratory infections in humans".

Community members also described the non-economic benefits of good animal welfare. They said that it is ethical and morally satisfying to give good care to animals. They felt guilty when animals experience physical pain and suffer from diseases or injuries. The community expressions about animal caregiving and handling showed how good animal welfare is important for their emotional well-being. In the study sites, community members stated that they become happy and feel better when they give good care to their animals. This close association and inter-dependence between animal welfare, livelihoods, and public health is an important reminder of how good animal welfare has both productivity as well as non-economic benefits for animal owners.

Ascribed animal welfare issues

During Community Conversations, community members identified the needs of their animals and the constraints to

meet those needs. A woman participant said, "if it is not for speaking, animals have similar needs and feelings as humans". Other participants said that "it is not only humans who need good things; animals also need good things". Animals need prevention and control of diseases, safe grazing, and control of parasite infections.

Women and men community members identified feed, salt, water, animal health, housing, and animal handling as common animal welfare issues. Describing the importance of sanitation (keeping animal shelters clean and dry), a male participant in Menz Gera said, "if you see dirty fleece, you can tell the sanitation in animal shelters". Similarly, a woman participant said, "the smell of the sheep can indicate the sanitation condition of animal shelters". Through the Community Conversations, community members recognized the consequences of poor animal welfare conditions. They explained that "when animal shelters are not clean, they can cause infections." They also indicated that "if animals do not get adequate feed, they will be emaciated, do not give enough milk and cannot resist or are susceptible to infectious diseases."

Community members indicated that they could observe animal behavior related to environmental conditions and "hear the voices" of their animals. They said that animals show behavioral responses such as reduced activity and responsiveness. Tail biting in dogs, vocalization of animals, running or unusual behaviors and feather pecking in poultry are behaviors induced by environmental inadequacies. These abnormal behavior patterns reflect inadequacies of the animal's environment or bad animal keeper behavior. Community members explained that behavioral observations related to feeding, drinking, or resting can give insights into the animal's feelings and requirements. They said that animals that are discomforted due to poor housing conditions, such as standing all night, show signs of injuries to their legs like staggering, stopping with one leg, or incoordination. Sick animals reduce their body weight. Fearful animals stay alarmed, run to other animals, or stand when approached.

Community members identified constraints to improving the welfare of their animals related to feeding, water, veterinary drugs, and service provision. They also described situations where animal handling could be improved. They stated that the shortage of feed and water critically affected the welfare of their animals. In Yabello, a male participant said, "we drive animals long distances on rough terrain in search of feed and water, which makes them exhausted or injured." Another participant said, "our animals get water in an interval of 2 or 3 days". Health-related constraints of animal welfare were the lack of veterinary clinics, veterinary drugs, and trained animal health workers. Community members indicated that the veterinary clinic was far from their village. The animal health workers were also not available all the time in the local veterinary clinic. Community members reported that animal health workers lived in town, and they were not accessible as they needed them. The veterinary clinic also lacked essential drugs and vaccines. As a result, community members often buy veterinary drugs from the market and administer the drugs by themselves or community animal health workers. They also indicated that vaccinations for common diseases were not available for all animal species, especially camels and equines.

Community actions

The Community Conversations aimed to not only identify and analyze animal welfare issues but also encourage community members to develop practical strategies to solve the issues along with local service providers. The community members set their vision for improved animal welfare and the actions that they thought should be taken (Table 3). The community action plans can contribute to improved human and animal welfare. Through Community Conversations, local service providers understood community issues and the community actions informed local planning processes, which can improve the capacity of both community members and local service providers to take actions toward improving the welfare of animals.

The local partners found the community dialogues engaging and empowering. The conversations helped create shared understanding (beyond individual learning) through social interaction and collaborative learning among community members and local service providers leading to the implementation of joint actions.

Innovative approaches such as putting women drawn from communities at the heart of animal welfare will achieve better results. The Community Conversations encouraged women and men community members to take ownership of animal welfare challenges and discuss solutions and think through their implementation, articulating the changes that they are likely to make. In Menz Gera, community members stated that the Community Conversations gave them a better understanding of animal welfare issues, and what it takes to meet the welfare of their animals. Both women and men community members recognized the importance of meeting the needs of their animals. A woman participant said, "the community discussions expanded our understanding of animal welfare issues".

Discussion

Through the Community Conversations, community members gained a multi-dimensional understanding of animal welfare. They described animal welfare as satisfying the biological functioning of animals, such as feed, water, shelter, and health care. However, it was not automatic for community members to identify the affective states and natural behaviors of animals in their view of animal welfare. Upon further in-depth discussion, community members recognized

TABLE 3 Community actions to improve animal welfare.

Priority animal welfare issues	Community actions	Expected benefits
Feed and water availability	• Timely collection of grass/haymaking/crop residues • Reduce herd size • Introduce improved forage production • Improve feeding and watering troughs • Improve grazing land management practices • Improve ration formulation of locally available feed resources • Pond construction and fencing for water points	• Increased feed and water availability • Happy, healthy, and productive animals • Saving animals' lives • Animals gain body weight • Good milk and butter production
Animal health management	• Regular vaccination and deworming • Construct animal health posts • Community mobilization based on scheduled vaccination/deworming programs • Improve animal housing sanitation by frequently cleaning barns • Buy veterinary drugs from approved sources • Monitor body and health condition of animals • Consult veterinarians when animals are sick • Report disease outbreaks timely	• Healthy, happy, and productive animals • Reduced cost of animal treatment • Reduced transmission of diseases to humans • Reduced effect of drug resistance
Animal handling practices	• Teach children not to hit animals • Hold household discussions about the effect of bad animal handling on the feelings and productivity of animals	• Happy and productive animals • Satisfaction of handlers

the feelings and natural behavior of animals as animal welfare components. Similarly, based on a semi-systematic review and thematic analysis of factors that influence farmers' views on farm animal welfare, Balzani and Hanlon (6) described three farmer categories according to their views on animal welfare. They showed that the biological functioning of an animal was the most common view of farmers, the affective state of an animal emerged as the second most common view, and the third category related to the ability of an animal to engage in natural behavior.

Community members also explored multiple benefits of good animal welfare, which are the drivers for their actions to improve the welfare of their animals. They described the welfare of their animals as being intertwined with their own livelihoods. While community members pronounced more on the economic benefits of good animal welfare (such as improved productivity of animals, saving on health costs, and increased incomes), they also acknowledged the non-economic benefits of good animal welfare (such as public health and psychological wellbeing of people). However, community members mostly described what the animal owners could benefit from good animal welfare, and they did not mention the benefits to the animals themselves. Similarly, using focus group discussions, Sinclair et al. (31) showed that economic and public health reasons (such as productivity, meat quality, food safety, human health, and livelihoods) were the most mentioned benefits of good animal welfare among livestock stakeholders across Asia and that improving animal welfare in the benefit of the animals themselves was not reported in most of the study countries.

The study shows that gender, age, and experience of animal owners seem to influence how they handle their animals. Previous studies (32–34) also found that individual characteristics such as the age and experience of the handler and cultural variables could influence human attitudes to animals and their welfare. Bad animal handling can cause stress and aggression both in animals and handlers. Hemsworth (35) showed that negative animal handling, such as beating animals harshly, shouting, and rapid movement, can make animals fearful, stressed, and unhappy. This can affect the health, welfare, and productivity of animals (13, 36). While farmers and pastoralists can recognize visible behavioral responses of their animals to negative handlings, such as animals showing fear, avoidance of humans, and refusing to feed, they may not recognize the psychological and physiological effects of negative handing on animals and their health, growth, productivity, and welfare. Through the conversations, community members recognized that good animal handling is as important as meeting the biological needs of their animals. The expressions community members used in describing animal welfare show that they have a sense of empathy for animals and their sense of responsibility and moral obligation for the good caregiving of their animals.

While animal beating is common by men and boys in highland areas (Menz Gera), in the pastoralist communities (Yabello), men reported good animal handling practices. This can be because of differences in the value systems, religious and cultural beliefs, and production systems of the communities. Animals, such as cattle, were more valued for production

purposes and animal handling was problematic in Menz Gera, for example, handling of plowing oxen. In contrast, social status or prestige was more important to pastoralists and animal handling was much better in Yabello. This was demonstrated in the songs community members sing their animals that express their values and relationships with their animals. This proximity of pastoralist communities with their animals like massaging, speaking to animals (calling animals by names), and singing songs to animals fosters empathy and is at the foundation of their understanding of animal welfare (6).

In the study sites, women tended to have more positive attitudes toward animals and are sensitive to the way they handle animals. This may be due to gender differences in empathy and values to animals (3). This may also be because women frequently handle milking cows (32) and develop more attachment to these animals than men, who frequently handle plowing oxen. Similarly, Campler et al. (33) showed that empathy attribute-related questions positively correlate with the gender of animal caretakers.

The understanding of community members' gendered perceptions of animal welfare and values for animals is important to inform gender-responsive animal welfare interventions. However, gender biases may be limiting service delivery and knowledge sharing both at the livestock extension service and community levels. While women have more positive attitudes to animals and animal welfare, gender norms and practices may limit their decision-making role in welfare improvement (21). Gender transformative approaches, such as Community Conversations, can support efforts to achieve both gender equality and animal welfare outcomes (37). The use of both single- and mixed-sex groups in the Community Conversations helped challenge community perceptions and influence their attitudes toward gender-equitable animal welfare management. Similarly, Lemma et al. (38) and Mulema et al. (22) showed that Community Conversations are supporting gender equality efforts in Ethiopia.

Understanding the constraints, risks, and opportunities of rural communities and the needs of the animals they care for can help improve both livelihoods and animal welfare outcomes. Given their gender roles in livestock management, women and men community members may have different risks and opportunities for improving the welfare of their animals and their own livelihoods. Women may be more exposed to zoonotic diseases (22, 39) and can be physically injured in handling animals. Animal owners' attitudes toward animals, their knowledge about giving care to animals, and resource and service constraints can limit their ability to improve the welfare of their animals (36). Animal welfare constraints are more prominent in small-scale and pastoralist farming systems, such as Yabello, where access to resources and livestock services is limited.

While community members demonstrate good knowledge of animal welfare and can identify where improvements could be made, there is a gap when it comes to addressing these issues. This gap extends to the veterinary support services that work with the farmers and pastoralists. As primary animal caregivers, community members need advice and training support to expand their knowledge and skills based on an understanding of their animal welfare perceptions, constraints, and needs (5, 40). This study and previous studies (22, 23) showed that the Community Conversations approach proved effective in strengthening the capacity of community members and local service providers to improve the welfare of animals in a gender-responsive manner.

Conclusion

The Community Conversations enabled community members and local service providers to better understand the multi-dimensional issues around animal welfare and how this can influence welfare improvement interventions. Community members described animal welfare as focusing on the biological needs of animals such as feed, water, and health, but there was also a good acknowledgment of the behavioral needs of animals as well as their ability to experience affective states. The community members identified feed, animal health, sanitation, and animal handling as priority animal welfare issues. There were also limitations in meeting the needs of animals both due to resource constraints, lack of knowledge, limited livestock services, and behavior of owners or caregivers. Changing the attitudes and practices of community members is critical for improving the welfare of their animals and their own livelihoods.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

Ethics statement

The studies involving human participants were reviewed and approved by the Institutional Research Ethics Committee of the International Livestock Research Institute. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

The concept of the Community Conversations on animal welfare was developed by ML, RD, and BW. Fieldwork was

conducted by ML, MM, and AK. A first draft of the manuscript was prepared by ML, expanded by RD, BW, and GA, then further revised by ML. All authors reviewed and approved the final version.

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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

References

1. Fraser D, Duncan IJ, Edwards SA, Grandin T, Gregory NG, Guyonnet V, et al. General principles for the welfare of animals in production systems: the underlying science and its application. *Vet J.* (2013) 198:19–27. doi: 10.1016/j.tvjl.2013.06.028

2. Fraser D, Weary DM, Pajor EA, Milligan BN. A scientific conception of animal welfare that reflects ethical concerns. *Anim Welf.* (1997) 6:187–205.

3. Cornish A, Raubenheimer D, McGreevy P. What we know about the public's level of concern for farm animal welfare in food production in developed countries. *Animals.* (2016) 6:74. doi: 10.3390/ani6110074

4. Weary DM, Robbins JA. Understanding the multiple conceptions of animal welfare. *Anim Welf.* (2019) 28:33–40. doi: 10.7120/09627286.28.1.033

5. Fuente SA, Caselli CB, Schiel N. People's perception on animal welfare: why does it matter? *Ethnobio Conserv.* (2017) 6:18. doi: 10.15451/ec2017106.1817

6. Balzani A, Hanlon A. Factors that influence farmers' views on farm animal welfare: a semi-systematic review and thematic analysis. *Animals.* (2020) 10:1524. doi: 10.3390/ani10091524

7. Doyle RE, Wieland B, Saville K, Grace D, Campbell AJD. The importance of animal welfare and Veterinary Services in a changing world. *Rev Sci Tech.* (2021) 40:469–81. doi: 10.20506/rst.40.2.3238

8. Global Alliance for Improved Nutrition. *Consumer and Vendor Perspectives on and Practices Related to Food Safety in Ethiopia: A Review*. (2022). A USAID EatSafe Project Report. Available online at: https://pdf.usaid.gov/pdf_docs/PA00Z882.pdf (accessed May 9, 2022).

9. Keeling L, Tunón H, Olmos AG, Berg C, Jones M, Stuardo L, et al. Animal welfare and the United Nations sustainable development goals. *Front Vet Sci.* (2019) 6:336. doi: 10.3389/fvets.2019.00336

10. FAO. *Livestock, Health, Livelihoods, and the Environment in Ethiopia. An Integrated Analysis*. (2019). Rome: FAO.

11. Asebe G, Gelayenew B, Kumar, A. The general status of animal welfare in developing countries: the case of Ethiopia. *J Veterinary Sci Techno.* (2016) 7:3. doi: 10.4172/2157-7579.1000332

12. Kauppinen T, Vainio A, Valros A, Rita H, Vesala KM. Improving animal welfare: qualitative and quantitative methodology in the study of farmers' attitudes. *Animal Welfare.* (2010) 19:523–36.

13. Jerlström J. *Animal Welfare in Ethiopia: Transport to and Handling of Cattle at Markets in Addis Ababa and Ambo. Degree Project in Animal Science.* Uppsala: Swedish University of Agricultural Sciences (SLU) (2013).

14. Lemma M, Mekonnen M, Tigabie A. *Community Conversation: An Approach for Collaborative Learning and Action in Animal Health Management*. Nairobi: ILRI (2021).

15. Central Statistical Agency [Ethiopia] and ORC Macro. *Ethiopia Demographic and Health Survey 2005*. Addis Ababa, Ethiopia and Calverton, Maryland: Central Statistical Agency and ORC Macro (2006).

16. Angassa A, Oba G. Herder perceptions on impacts of range enclosures, crop farming, fire ban and bush encroachment on the rangelands of Borana, southern Ethiopia. *Hum Ecol.* (2008) 36:201–15. doi: 10.1007/s10745-007-9156-z

17. Wako G, Tadesse M, Angassa A. Camel management as an adaptive strategy to climate change by pastoralists in southern Ethiopia. *Ecol Proc.* (2017) 6:26. doi: 10.1186/s13717-017-0093-5

18. Shapiro BI, Gebru G, Desta S, Negassa A, Nigussie K, Aboset G, Mechale H. *Ethiopia Livestock Sector Analysis: A 15-Year Livestock Sector Strategy*. ILRI Project Report. Nairobi: ILRI (2017).

19. FAO. *The Future of Livestock in Ethiopia. Opportunities and Challenges in the Face of Uncertainty*. Rome: FAO (2019).

20. Kinati W, Mulema A. Gender issues in livestock production systems in Ethiopia: A literature review. *J Livestock Sci.* (2019) 10:66–80. doi: 10.33259/JLivestSci.2019.66-80

21. Lemma M, Gizaw S, Etafa A, Mulema AA, Wieland B. *Gender Integration in the Ethiopian Agricultural Extension System: A Literature Review*. Nairobi: ILRI (2020).

22. Mulema AA, Kinati W, Lemma M, Mekonnen M, Alemu BG, Elias B, et al. Clapping with two hands: transforming gender relations and zoonotic disease risks through community conversations in rural Ethiopia. *Hum Ecol.* (2020) 48:651–63. doi: 10.1007/s10745-020-00184-y

23. Lemma M, Kinati W, Mulema AA, Mekonnen M, Wieland B. *Community Conversations: A Community-Based Approach to Transform Gender Relations and Reduce Zoonotic Disease Risks*. Nairobi: ILRI (2019).

24. Wals, AEJ, van der Hoeven, EMMM, Blanken H. *The Acoustics of Social Learning: Designing Learning Processes That Contribute to a More Sustainable World*. Wageningen: Wageningen Academic Publishers (2009).

25. Biggs S, Matsaert H. *Strengthening Poverty Reduction Programs Using an Actor-Oriented Approach: Examples From Natural Resources Innovation Systems*. Network Paper No.134. ODI: Agricultural Research and Extension Network (2004). Available online at: https://cdn.odi.org/media/documents/8260.pdf (accessed May 12, 2022).

26. German L, Stroud A. A framework for the integration of diverse learning approaches: operationalizing agricultural research and development (R&D) linkages in Eastern Africa. *World Dev.* (2007) 35:792–814. doi: 10.1016/j.worlddev.2006.09.013

27. MacDonald C. Understanding participatory action research: a qualitative research methodology option. *Can J Action Res.* (2012) 13:34–50. doi: 10.33524/cjar.v13i2.37

28. Doyle R, Lemma M, Mulema A, Wieland B, Mekonnen M. *Community Conversation on Animal Welfare: A Guide to Facilitators*. Nairobi: ILRI (2019).

29. Lemma M, Mekonnen M, Kumbe A, Demeke Y, Wieland B, Doyle R. *Community Conversations Report on Animal Welfare*. Nairobi: ILRI (2019).

30. Nowell LS, Norris JM, White DE, Moules NJ. Thematic analysis: striving to meet the trustworthiness criteria. *Int J Qual Methods.* (2017) 16:1–13. doi: 10.1177/1609406917733847

31. Sinclair M, Fryer C, Phillips CJC. The benefits of improving animal welfare from the perspective of livestock stakeholders across Asia. *Animals.* (2019) 9:123. doi: 10.3390/ani9040123

32. Garsow AV, Biondi MR, Kowalcyk BB, Vipham JL, Kovac J, Amenu K, et al. Exploring the relationship between gender and food safety risks in the dairy value chain in Ethiopia. *Int Dairy J.* (2022) 124:105173. doi: 10.1016/j.idairyj.2021.105173

33. Campler MR, Pairis-Garcia MD, Rault JL, Coleman G, Arruda AG. Caretaker attitudes toward swine euthanasia. *Transl Anim Sci.* (2018) 2:254–62. doi: 10.1093/tas/txy015

34. Serpell JA. Factors influencing human attitudes to animals and their welfare. *Anim Welf.* (2004) 13:1 45–51.

35. Hemsworth P. Human-animal interactions in livestock production. *Appl Anim Behav Sci.* (2003) 81:185–98. doi: 10.1016/S0168-1591(02)0 0280-0

36. Martínez GM, Suárez VH, Ghezzi MD. Influence of the human-animal relationship on productivity and animal welfare in dairy farms. *Dairy and Vet Sci J.* (2019) 11:555825. doi: 10.19080/JDVS.2019.11.5 55825

37. Drucza K, Abebe W. *Gender Transformative Methodologies in Ethiopia's Agricultural Sector: A Review*. Addis Ababa: CIMMYT (2017).

38. Lemma M, Kinati W, Tigabie A, Mekonnen M. *Community Conversations Empower Women and Transform Gender Relations in Rural Ethiopia*. Innovation Brief. Addis Ababa: ICARDA (2021).

39. Wieland B, Alemu B, Desta H, Kinati W, Mulema AA. *Participatory Epidemiology and Gender Analysis to Address Small Ruminant Disease Constraints in LIVESTOCK and Fish and AfricaRISING Project Sites in Ethiopia.* (2016). Available online at: https://cgspace.cgiar.org/bitstream/handle/10568/76685/LF_peg_jul2016.pdf (accessed June 3, 2022).

40. Atkinson O. Communication in farm animal practice 1. Farmer–vet relationships. *In Pract.* (2010) 32:114–7. doi: 10.1136/in p.c836

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An exploratory study on differences in maternal care between two ecotypes of Nigerian indigenous chicken hens

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The Yoruba (YRE) and Fulani (FLE) are the two notable indigenous chicken ecotypes in Nigeria. They exhibit broodiness and post-hatch care of their chicks. Studies on welfare, productivity, and maternal behaviors of these two ecotypes are scarce, hence the need for this study. Separate flocks of these ecotypes were housed intensively and hens that showed broodiness (ten YRE and five FLE) were monitored. Brooding behaviors were monitored for 3 days in the 1st and 2nd weeks of brooding and daily in the 3rd week of brooding for 6 h/day (07:00–09:00 h, 11:00–13:00 h, and 15:00–17:00 h). During brooding, surface body temperatures (eye, brood patch and under the wings), egg temperature and body weight of the hens were measured. Chicks hatched (44 chicks from the YRE and 24 chicks from the FLE) by these hens were subjected to tonic immobility tests on the 7th, 14th, and 21st days post-hatch and to a simulated predator test on the 8th, 15th, and 22nd days post-hatch to determine their level of fear. In each ecotype, brooding behaviors did not change over the three weeks, but the YRE hens spent longer time sitting on their eggs at the 2nd ($U = 5.000$, $z = -2.454$, $P = 0.014$) and 3rd ($U = 9.000$, $z = -1.961$, $P = 0.050$) week of brooding. The surface body temperatures of both ecotypes, egg temperature, and relative weekly weight loss were similar over the brooding period, but relative weekly weight loss was greater ($P < 0.05$) at the 3rd than 1st and 2nd week of brooding. The surface body temperatures were positively correlated ($P < 0.01$) with egg temperature. In both ecotypes, attempts to induce and duration of tonic immobility were similar over the test periods but on the 7th day post-hatch, the duration of tonic immobility was longer ($U = 323.000$, $z = -2.632$, $P = 0.008$) and on the 14th day post-hatch, the number of attempts to induce tonic immobility was less ($U = 332.000$, $z = -2.630$,

$P = 0.009$) in the YRE chicks. In conclusion, YRE hens sat more on the eggs and their chicks were more fearful.

KEYWORDS

behavior, broodiness, ecotype, fear, maternal care, Nigerian indigenous chickens

Introduction

In developing and underdeveloped countries, indigenous chickens are more abundant, with Nigeria having the most among the Sub-Saharan countries (1). Nigerian indigenous chickens (NICs) are found in several geopolitical zones around the country and are classified according to genetic lines of feathering (normal feather, naked neck, and frizzle feather), color variants (black, white, brown, and mottled), and ecotypes [Yoruba (YRE) and Fulani (FLE)] (2, 3). Both ecotypes are good scavengers and have excellent immunity against endemic diseases (4). They are known for their hardiness, adaptability and survivability (5).

According to the Food and Agriculture Organization (6), ecotype refers to a population within a breed that is genetically adapted to a specific habitat. The natural habitat where these two chicken ecotypes are prevalent differs. The FLE is found in the dry savannahs (Guinea and Sahel savannah) while the YRE is found in the forest zones (7). However, due to the settlement of Fulani herdsmen and their families in the forest zone, where they can get forage for their cattle, they brought their chickens (FLE) with them. Presently, there is an increasing population of FLE in the forest zone. Some people in the Southwest part of Nigeria prefer to buy and raise the FLE over the YRE ecotypes, probably because of their bigger size for more meat, higher egg production and better feed conversion.

There are reports on the differences between the YRE and FLE in terms of body weight, body structure, and egg production capacities, but little is known about their maternal behavior. The FLE weighs between 1.2 and 2.0 kg at maturity (8–10) while the YRE weighs between 0.68 and 1.50 kg at maturity (11). Based on body structure, the FLE and YRE are referred to as the "heavy ecotype" and "light ecotype", respectively (12) (Figure 1). The chest circumference, wingspan, beak length, tarsometatarsus length, and body length of the FLE are greater than the YRE (13). In terms of egg production, the YRE lays earlier (20–23 weeks) than the FLE (22–31 weeks), but the FLE lays bigger, and more eggs compared to the YRE (14). These chicken ecotypes can serve as a rapid means of bridging protein deficiency and providing an additional source of income to the livelihoods of low-income families in urban, peri-urban, and rural settlements (15). Thus, these chickens play major roles in rural economies and contribute significantly to the Gross National Product of Nigeria (16).

Since these two chicken ecotypes are reared under the scavenging system, selecting an ecotype with good productivity and mothering abilities will benefit the poultry industry. In the first, second, and third weeks of brooding, YRE hens spent 88–93% and 0.06–0.11% of their time sitting on the eggs and engaging in ingestive behavior, respectively (17). The YRE hens showed behavior indicative of distress (increased pacing) when separated from their chicks visually rather than physically (18).

The indigenous chickens still exhibit their full natural behavior repertoire, which is very important to animal welfare (19). However, genetic selection for increased egg production in commercial laying hens has eliminated broodiness (20), which means that these hens can neither incubate eggs nor hatch chicks by themselves. Although chicks are precocial animals, they still require maternal care, especially in the first few weeks of life (21), to survive in the natural environment. In commercial poultry production, chicks can survive without their mothers, but this comes with several welfare issues. Rearing without a mother hen has major effects on the chicks' behavioral development (22). Brooded birds are less fearful at a young age (23), show greater exploratory behavior in a new environment (24, 25), and display less feather pecking and cannibalism, resulting in lower mortality rates compared to non-brooded birds (26).

Commercial laying hens have serious welfare issues such as feather pecking and cannibalism. The occurrence of this behavior has been linked to the lack of maternal care in early life. Hewlett and Nordquist (27) found no effect of maternal care in a commercial hybrid line of layer hen (a cross between White Leghorn and the Brown Nick), probably because the selection process has impaired the response of these chicks to maternal care. The style of maternal care adopted in their study was a cross-fostering type (using a Silkie Bantam hen to foster the commercial laying chicks). Both chicken breeds have different behavioral repertoires and welfare issues. The hybrid layer chicks already have their own innate behavior which they have inherited from their parents, which is different from that of the foster mother.

Although commercial strains may not show maternal care when reared by mothers, even after 45 weeks of removal from the mothers, the hens showed changes in brain structures (an increase in arginine vasotocin neurons in the medial pre-optic area of the hypothalamus), suggesting that they were receptive to maternal care. This indicates that commercial strains can only benefit from maternal care but cannot be maternal caregivers

(28). Maternal caregiving can be found only in chickens that have not been subjected to genetic selection for increased egg productivity.

With the increasing impact of climate change on animal welfare, selecting an ecotype that is already adaptable to the tropical environment with high production capacity to meet the required protein needs (egg and meat) of the Nigerian population is needed. So, we aimed to identify the ecotype with better mothering abilities to raise chicks of good welfare with the potential to escape from predators and survive in the natural environment or free-range housing system. To achieve this aim, we assessed the brooding behavior of the two ecotypes and the longer-term effects of maternal care on fear of the offspring of these ecotypes using the conventional tonic immobility (TI) and a simulated predator test. We also examined whether the fear level of the chicks increased as they age. We hypothesized that there would be differences in the brooding behavior of the two ecotypes due to the differences in their genetic make-up which has conferred on them different body sizes, structure, and productivity. This in turn will reflect in some behavioral differences in the fear level of their chicks.

Materials and methods

Experimental site

The experiment was carried out at the Poultry Unit of the Directorate of University Farms (DUFARMS), Federal University of Agriculture, Abeokuta (FUNAAB). The University is located on latitude 7°10'N, longitude 3°2'E, and altitude 76 m above sea level. The area lies in the Southwestern part of Nigeria and has a prevailing tropical climate with a mean annual rainfall of 1,037 mm and an annual mean temperature and relative humidity of 28°C and 82%, respectively.

Experimental birds and management

All procedures in this study were based on guidelines of the Animal Care and Use Committee of the Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. Thirty hens and five cocks per ecotype (YRE and FLE) were selected for this study. The FLE cocks and hens used in this study had an average weight of 2 and 1.2 kg, respectively, while the YRE cocks and hens had an average weight of 1.4 and 0.8 kg, respectively.

The two ecotypes were housed each in five separate deep litter floor pens (3 × 5 m) littered with 5 cm of wood shavings. In each replicate pen, a cock and six hens were housed and provided with a perch (111 cm high), nest boxes (30 × 30 cm), and sand-bath (76 × 76× 5 cm) that allowed the birds to perform their natural behaviors. The FLE chickens were obtained from a Fulani settlement at Kishi, Oyo State, Nigeria, and allowed to acclimatize for a month before the commencement of the experiment. The YRE chickens were obtained from an already existing flock at the research station. Once broodiness was confirmed (continuous sitting on eggs), the hens were separated into brooding (BRD) pens (similar in size to their home pens) and 10 eggs (laid by hens of the same ecotype) were placed underneath them in a nest box. The nest boxes were bedded with 2 cm of wood shavings to prevent the eggs from breaking. All the birds were provided with ready-made layer mash having the following composition: 16.5% CP, 2,725–2,980 kcal/kg metabolizable energy, 5% fat/oil, 6% crude fiber, 3.60% calcium, 0.45% available phosphorus, 0.80% lysine, 0.34% methionine and 0.30% salt. Birds were fed this compounded feed at 120 g/bird/day and water was provided *ad libitum*.

Experimental procedure

Surface body and egg temperatures

The surface body temperatures (SBTs) of the broody hens were measured three times a week and their body weights were measured weekly. The SBTs of the hens were measured from three body parts (eye, under the wing, and brood patch) using a non-contact infra-red thermometer (Model: IT-122, accuracy ± 0.2°C, made in China). Also, the temperature of the eggs (EGT) was measured using an infra-red thermometer and the average egg temperature was calculated.

Brooding behavior

The behaviors of the brooding (BRD) hens (10 YRE and 5 FLE) were recorded for three weeks. Each BRD hen was monitored three times weekly during the first two weeks of BRD, and then daily during the last week of BRD for a total of six hours/day (morning = 07:00–09:00 h, afternoon = 11:00–13:00 h, and evening = 15:00–17:00 h) using CCTV cameras (Winposse, Model: WP-F6036TP-H, lens 3.6 mm, made in China) with 2.0 Megapixels, positioned to cover the entire pen. The behaviors of interest include sitting on the egg, turning of eggs, feeding, drinking, vigilance with eyes open, and eyes close while sitting on the eggs, as described in Table 1. After hatching, the nest box, unhatched eggs, and broken shells were removed from the pen, and the hen and her chicks were left in the same pen until the fourth-week post-hatch (PTH) when the chicks were weaned. The chicks were provided with chick mash (CP = 21%, metabolizable energy of 3,000 kcal/kg) in chick tray feeders (diameter 20 cm) and water in bell drinkers (diameter of 21.50 cm, 2-l capacity). Each chick was wing-tagged after hatching for easy identification.

TABLE 1 Behavioral categories and description.

Behavior category	Description
Sitting on eggs	Hen sitting continuously on the egg
Turning of eggs	Hen turns the egg with her beak intermittently or moves her body gently against the egg
Feeding	Hen leaves nesting position and directs its beak into the feed trough and starts pecking at the feed
Drinking	Hen leaves nesting position and directs its beak into the bowl drinker to drink water
Eyes open while sitting on eggs	Hen maintains nesting position with the eye opened
Eyes close while sitting on eggs	Hen maintains nesting position but intermittently closes the eye

Tonic immobility test (TI)

The level of fear in the chicks was measured using the TI test. To assess the level of fear, forty-four YRE and twenty-four FLE chicks were tested at each time (7, 14, and 21 st-day PTH) between 9:00 and 11:00 h. The chicks were chosen at random from their mothers and tested individually in a separate test room within the same poultry house by restraining them for 15 s with one hand on the sternum and the other on the head and placing them on a table. Then both hands were released. The variables observed were the number of attempts to induce TI and the latency of the bird to righting itself i.e., duration of TI was recorded with a stopwatch (maximum duration was 5 mins). If the immobility duration was <10 s before the chick righted itself, the induction was considered unsuccessful and the chick was subjected to another TI test and the number of attempts was recorded. Longer durations of TI are interpreted as indicating a higher level of fear (29). Immediately after testing each chick, it was returned to its mother and the next chick was picked.

Predator test

The simulated predator (plastic dinosaur, Figure 2) was hung halfway from the top of the test arena (88 × 116 × 138 cm) before introducing each chick into the test arena. Once the chick was placed inside the test arena, the door was locked and then the experimenter from outside pulled the rope to which the predator was hung so that it began to swing and the red lights on the simulated predator were lit by pressing a remote. The predator test was undertaken on the 8th, 15th and 22nd days PTH on forty-four YRE and twenty-four FLE chicks. The immediate reaction of each chick was monitored with a CCTV camera positioned inside the test arena for 5 min. The behavior of the chicks was scored on a scale of 1 (not fearful) and 2 (fearful). The behavior of chicks categorized as "not fearful" was when there was no visible change in the chicks' behavior. Chicks were scored as "fearful" when they showed any of freezing, crouching, or running behavior.

Data analysis

A normality test (Shapiro-Wilk) was performed on the collected data, but none of the data on brooding behavior and fear was normally distributed. So, we used the non-parametric repeated measure analysis, Friedman test, to analyze the behavior of the hens during brooding (three weeks of brooding) and the behavior of their chicks during the tonic immobility test for the three-time points (day 7, 14, and 21 post-hatch). Since the Friedman Test does not allow a between-subject factor (which is ecotype in this case), we analyzed the data using the Friedman test separately for each ecotype and corrected for multiple comparisons using the Bonferroni correction (since we had three timepoints, so significance was based on $P < 0.017$ i.e., 0.05/3 and not on $P < 0.05$). The effect of ecotype on BRD behavior and TI was analyzed using the Mann-Whitney U test at each time point. The behavior of the chicks during the predator test was categorized as either "not fearful" or "fearful". The effect of ecotype on behavior during the predator test was analyzed using descriptive statistics and inferential statistics. Data from the three body surfaces and egg temperatures were normally distributed and were analyzed using a repeated measures ANOVA having time points (week 1, 2, and 3 post-hatch) as the within-subject factor and ecotype (YRE and FLE) as the between-subject factors. If Mauchly's test of sphericity was significant, then we used Greenhouse Geisser. A Pearson's correlation was undertaken to establish the relationship between the surface body temperatures of the broody hens and the average temperature of their eggs. All statistical analysis was undertaken using the SPSS statistical package (version 23) except for the inferential statistics of the predator test, which was analyzed using the GENMOD procedure of SAS (version 9.4) with binomial distribution and Probit link function.

Results

Brooding

Behaviors of the hens

For both hen ecotypes, the proportion of time spent by the hens sitting on the eggs, egg turning, feeding, drinking, and eyes open or close while sitting on the eggs did not differ ($P > 0.05$) over the three weeks of BRD (Figures 3–8). However, the proportion of time spent sitting on the egg was greater in the YRE at the 2nd ($U = 5.000$, $z = -2.454$, $P = 0.014$) and 3rd

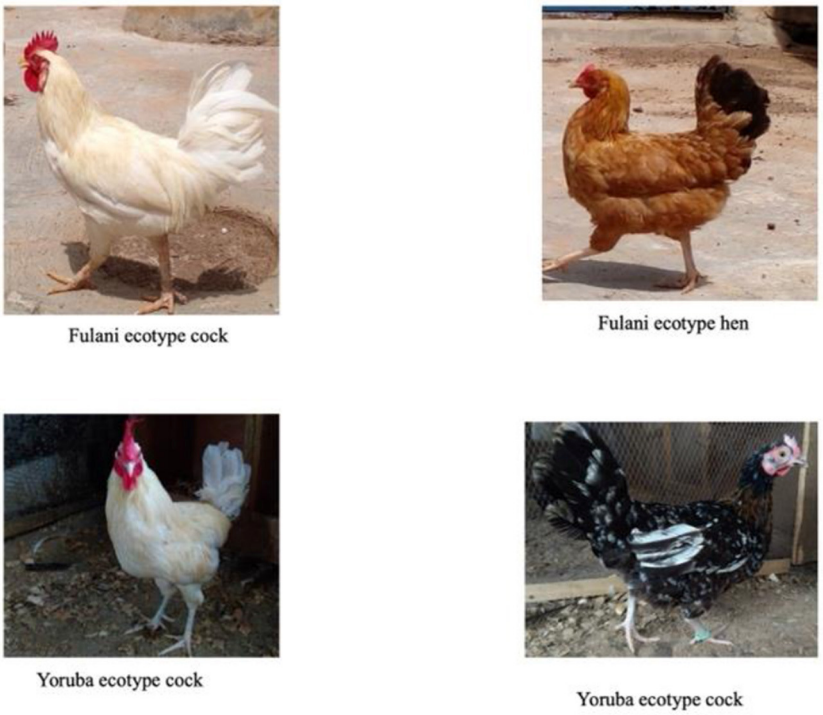

FIGURE 1
Fulani and Yoruba ecotype chickens.

($U = 9.000$, $z = -1.961$, $P = 0.050$) weeks of BRD than in FLE hens (Figure 3). Ecotype had no significant effect ($P > 0.05$) on the other BRD behaviors (Figures 4–8).

Body surface temperatures of the broody hen

There was no significant effect ($P > 0.05$) of the week of brooding, week × ecotype, and ecotype on the eye, wing, and brood patch temperatures of the two hen ecotypes (Table 2).

Temperature of the brooded eggs

There were no significant effects ($P > 0.05$) of the week, week × ecotype, and ecotype on the temperatures of the broody hens' eggs (Table 2). There were positive correlations ($P < 0.01$) between all three body surface temperatures and the temperature of the brooded eggs (Table 3).

Relative weekly weight loss

There was a significant effect of week of brooding ($F_{1.254,16.302} = 8.743$, $P = 0.006$) on the relative weekly weight loss which was greater at the 3rd than the 1st and 2nd weeks of brooding. There was no significant ($P > 0.05$) week × ecotype interaction and the main effect of ecotype on relative weekly weight loss (Table 4).

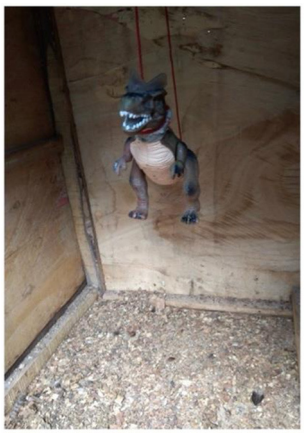

FIGURE 2
Simulated predator hung in the test arena.

Post-hatch fear behaviors in the two chick ecotypes

Tonic immobility test

Results from the repeated measures analysis showed that the number of attempts to induce tonic immobility and the

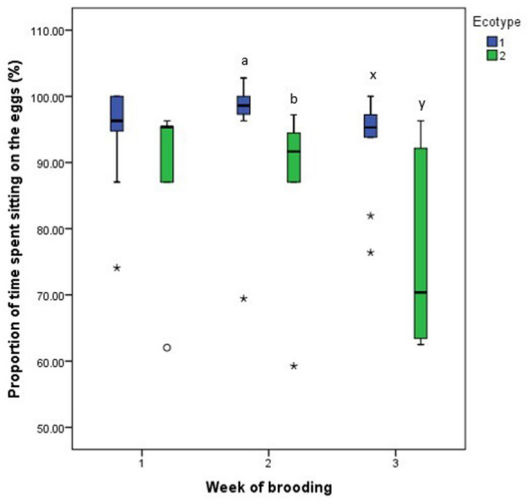

FIGURE 3
Proportion of time spent by two Nigerian indigenous hen ecotypes (1 = Yoruba and 2 = Fulani) sitting on the eggs over the three weeks of brooding. [ab]Means differ at $P < 0.05$ at week 2 and [xy]Means differ at $P < 0.05$ at week 3. Outliers in the data are depicted by the symbol "* or ◦".

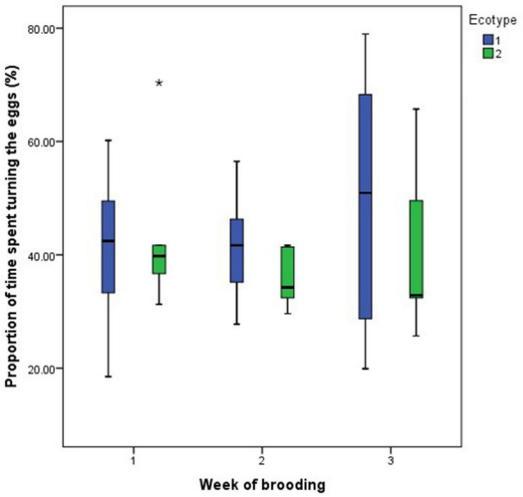

FIGURE 4
Proportion of time spent by two Nigerian indigenous hen ecotypes (1 = Yoruba and 2 = Fulani) turning the eggs over the three weeks of brooding. Outlier in the data is depicted by the symbol "*".

duration of tonic immobility for each of the ecotypes was similar across the three-time points (days 7, 14, and 21 PTH), Figures 9, 10. Further analysis of the effect of ecotype showed that on the 14th day PTH, the number of attempts to induce tonic immobility was less ($U = 332.000$, $z = -2.630$, $P = 0.009$, Figure 9) in the YRE than in the FLE chicks. On the 7th-day PTH, the duration of tonic immobility was longer ($U = 323.000$, $z = -2.632$, $P = 0.008$, Figure 10) in the YRE than in the FLE chicks.

Predator test

Although the inferential statistics revealed no effect of ecotype on the fear score on the 8th, 15th, and 22nd PTH days, the descriptive statistics show some interesting trends (Figure 11). The result showed two distinct fear responses in the chicks: the "not fearful" and the "fearful" categories. The percentage of chicks that showed no fear response to the simulated predator was similar in the two ecotypes on the 8th day PTH, but on the 15th and 22nd -day PTH, the percentage of YRE chicks seemed to increase and seemed to be greater than the FLE chicks. On the other hand, among the chicks that showed a higher fear response to the simulated predator (fearful category), on the 8th-day PTH, there was a similar percentage of YRE and FLE chicks. However, on the 15th and 22nd days PTH, the percentage of YRE chicks was reduced and the FLE chicks increased. Overall, there seemed to be a greater number of chicks that belonged to the "fearful" category than to the "not fearful" category.

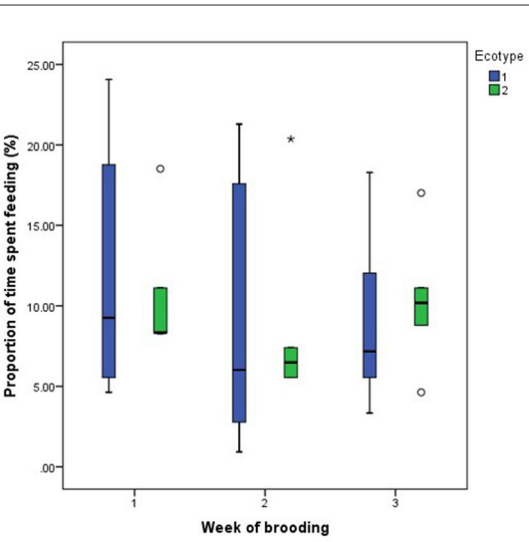

FIGURE 5
Proportion of time spent by two Nigerian indigenous hen ecotypes (1 = Yoruba and 2 = Fulani) feeding over the three weeks of brooding. Outliers in the data are depicted by the symbol "* or ◦".

Discussion

The aim of this study was to investigate the potential of having a tropically adapted chicken breed with high productivity to meet the protein needs of the Nigerian population and have good maternal care to raise offspring with fewer welfare issues

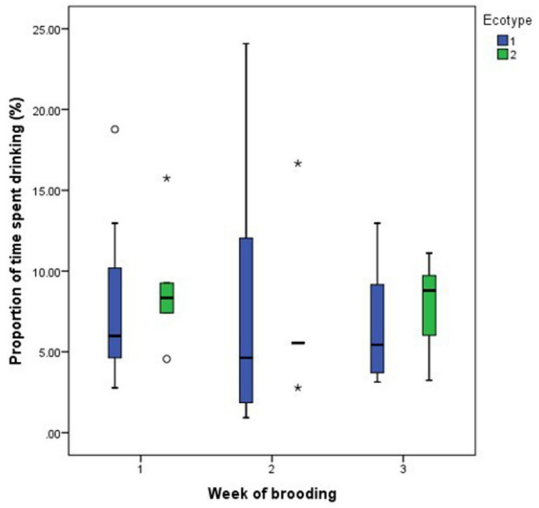

FIGURE 6
Proportion of time spent by two Nigerian indigenous hen ecotypes (1 = Yoruba and 2 = Fulani) drinking over the three weeks of brooding. Outliers in the data are depicted by the symbol "* or ◦".

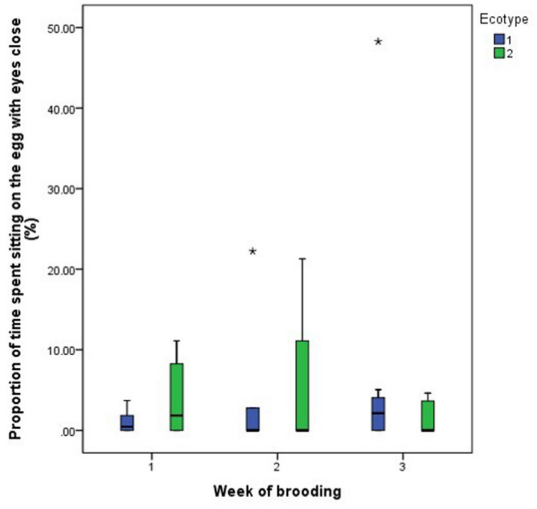

FIGURE 8
Proportion of time spent by two Nigerian indigenous hen ecotypes (1 = Yoruba and 2 = Fulani) sitting on the eggs with eyes close over the three weeks of brooding. Outliers in the data are depicted by the symbol "*".

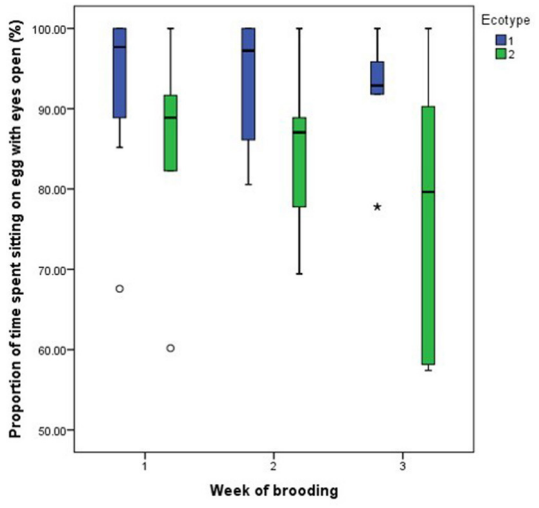

FIGURE 7
Proportion of time spent by two Nigerian indigenous hen ecotypes (1 = Yoruba and 2 = Fulani) sitting on the egg with eyes open over the three weeks of brooding. Outliers in the data are depicted by the symbol "* or ◦".

and the ability to survive in a free-range rearing system based on their ability to display appropriate fear responses when they encounter real-time predators.

It was our intention to have a minimum of 15 broody hens per ecotype, but within the 7-month experimental period (July 2021 and January 2022), only 10 out of 30 (33.3%) YRE and 5 out of 30 (16.7%) FLE hens became broody. A previous study by Iyasere et al. (17) on BRD behavior, reported a 30% success of the YRE becoming broody in a similar intensive rearing system. The breakdown of when the hens became broody in the current study is as follows; July (three YRE and one FLE), September (three YRE), October (two YRE and one FLE), November (two YRE), and December (three FLE). The reason for the low number of FLE hens that became broody within the period of this study could indicate that they needed more time to get acclimatized to the intensive conditions at our research station. We sourced the FLE chickens from Fulani people that settled in a village in Kishi, Irepo Local Government Area of Oyo State, where they are raised under the extensive system. There could also be the possibility of the season affecting the broodiness of the FLE hens because three out of the five FLE hens that became broody were recorded in December which falls in the early dry season of the year in Nigeria. The FLE chickens originated from Northern Nigeria, so their breeding season may be favored by hot or dry weather. Further studies are required to investigate the influence of acclimatization and season on broody hens in these two chicken ecotypes.

It is also worth mentioning that the low number of broody hens could be due to the fact that we adopted a natural broody method in the current study where eggs were left in the nest boxes and hens were exposed to natural daylight (12L:12D). Other studies have induced broodiness by extending the daylight to 16 h in addition to the provision of eggs in the nest box, which resulted in a 46.7% success in the Silkie and Wyandotte hens (23, 30). In order to

TABLE 2 Surface body temperatures of the two ecotypes (Yoruba, YRE, and Fulani, FLE) of broody hens and the average temperature of their eggs for the three weeks of the brooding period.

Temperatures	Week 1		Week 2		Week 3	
	YRE	FLE	YRE	FLE	YRE	FLE
Eye temperature (°C)	35.67 ± 0.64	36.23 ± 0.91	35.51 ± 0.53	36.61 ± 0.75	35.94 ± 0.56	36.34 ± 0.80
Wing temperature(°C)	35.67 ± 0.63	36.54 ± 0.89	35.42 ± 0.56	36.75 ± 0.79	35.82 ± 0.67	36.04 ± 0.95
Brood patch temperature (°C)	36.38 ± 0.41	36.82 ± 0.58	36.30 ± 0.38	37.60 ± 0.54	36.70 ± 0.40	37.05 ± 0.57
Egg temperature (°C)	35.51 ± 0.51	35.94 ± 0.73	35.47 ± 0.52	36.13 ± 0.73	35.93 ± 0.52	35.46 ± 0.73

Values are Means ± SEM.

TABLE 3 Pearson's correlation between surface body temperatures of the broody hens and the temperature of the brooded eggs.

	Eye temperature	Wing temperature	Brood patch temperature	Egg temperature
Eye temperature	1.000	0.971**	0.881**	0.951**
Wing temperature		1.000	0.867**	0.945**
Brood patch temperature			1.000	0.824**
Egg temperature				1.000

**$P < 0.01$.

increase the number of broody hens in future studies, we may consider the extension of daylight after first investigating whether induction has no negative welfare implications for the hen.

In the current study, each ecotype showed no difference in all the BRD behaviors monitored over the three weeks. This implies that once BRD commenced, the hens' behaviors remained consistent irrespective of the stage of development of the embryo, until the chicks hatch. Iyasere et al. (17) also observed consistent sitting on eggs and ingestive behavior in the YRE ecotype over the three weeks of BRD. Broodiness is controlled by the prolactin hormone (31). Behaviorally, the most obvious sign of BRD in a hen is continuous sitting in the nest box whether on eggs or not, and emitting a "growling sound" and puffing of feathers when approached. Other behavioral changes include reduced feed and water intake, turning and retrieval of eggs, aggressive or defensive behaviors, and cessation of egg-laying (32). As heat is transferred from the hen through the brood patch to the eggs for the development of the embryo, it is very important that the hen turns the egg at intervals to ensure uniform development of the embryo and prevention of embryo from sticking to the shell (33).

The reduction in feeding activities during BRD causes the hens to lose weight. The higher relative weight loss in both hen ecotypes at the 3rd week of BRD could be attributed to a greater depletion of body reserves required to maintain the heat production needed for the development of the embryo (34). We observed a 7.64 and 7.21% relative weight loss over the three weeks in the YRE and FLE ecotypes, respectively. Brooding pheasant hens lose weight from almost all body tissues and organs (35).

TABLE 4 Relative weight loss (%) of the broody hens over the three-week brooding period.

Ecotype	Week 1 (%)	Week 2 (%)	Week 3 (%)
Yoruba (YRE), n=10	-1.68 ± 0.18^{b}	-2.18 ± 0.20^{b}	-3.78 ± 0.64^{a}
Fulani (FLE), n=5	-1.73 ± 0.25^{b}	-1.60 ± 0.28^{b}	-3.44 ± 0.90^{a}

Values are Means ± SEM, ^{ab}Means differ at $P < 0.05$.

In the current study, both hen ecotypes were provided with ten eggs each to incubate once broodiness was confirmed. The longer time spent sitting on the eggs by the YRE hens in the 2nd and 3rd weeks could be due to two main reasons. Firstly, the YRE hens may need extra effort to accommodate the large number of eggs placed underneath them since they have smaller chest dimensions, which easily accommodate their small clutch size of 2–6 eggs, compared to those of the FLE hens, with bigger chest dimensions to accommodate a bigger clutch size of 3–9 eggs (14). Secondly, the YRE eggs have a thicker eggshell (5.12 mm) compared to the FLE (4.89 mm) eggs (14), so more effort may be required from the hen to generate the needed heat to penetrate this thick shell for the development of the embryo.

In a comparative study on the effect of body size of Bangladesh broody hens on hatchability and chick survival, it was observed that Bangladesh broody hens with an average body

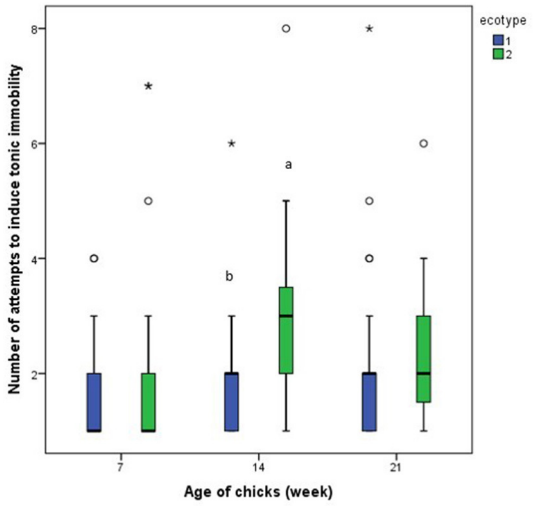

FIGURE 9
Number of attempts to induce tonic immobility in two ecotypes (1 = Yoruba and 2 = Fulani) of Nigerian indigenous chicks at days 7, 14, and 21 post-hatch. [ab]Means differ at $P < 0.05$ at day 14 post-hatch. Outliers in the data are depicted by the symbol "* or ◦".

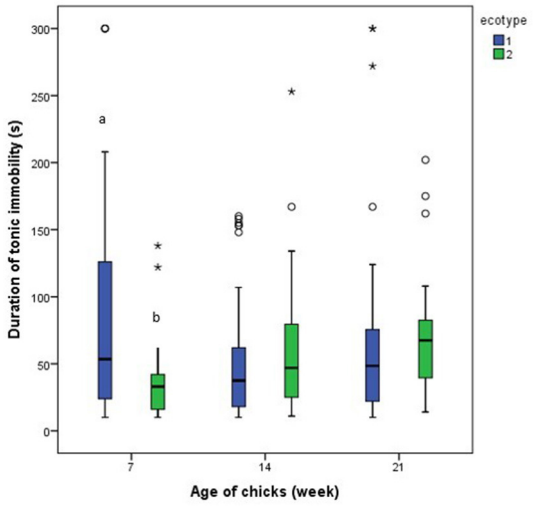

FIGURE 10
Duration of tonic immobility in two ecotypes (1 = Yoruba and 2 = Fulani) of Nigerian indigenous chicks at days 7, 14, and 21 post-hatch. [ab]Means differ at $P < 0.05$ at day 7 post-hatch. Outliers in the data are depicted by the symbol "* or ◦".

size of 800–950 g were able to hatch 87.2% of the eggs when provided with 17 eggs to incubate, each with an average weight of 41 g (36). However, there was no report on whether the body size of the hens influenced their BRD behavior. The inability of the hens to hatch the remaining 12.8% could be that their small body size could not accommodate all the eggs underneath them. For a hen with a body size of 800–950 g, Azharul et al. (36) recommended placing 14 eggs for incubation. The YRE hens used in the current study have an average body weight of 828 g, which is close to that reported in the Bangladesh broody hens, so the YRE may not have the capacity to incubate as many eggs as the Bangladesh hens.

Broody hens sit on their eggs to provide the heat which is transferred from their bodies, especially the chest/breast region or brood patch, to the eggs. This corroborates our observation of both ecotypes having similar SBTs (eye, wing, and brood patch) and EGT, as the developing embryo is very sensitive to temperature changes. Interestingly, we observed positive correlations between the SBTs of the hen and the temperature of the eggs she was brooding. In addition, the current study showed that the SBTs of the hens of both ecotypes were similar over the three-week BRD period. This implies that the hens were able to maintain their body temperatures at a level that was appropriate for the development of the embryo. Iyasere et al. (17) previously reported that the rectal temperature of the YRE hens remained constant over the three weeks of BRD, but the breast temperature was higher during the first and second weeks than during the third week of BRD. The reason for this inconsistency could be related to the robust data available in the current study (hens' SBTs were measured three times a week and the average calculated per week), but a single measurement per week was taken in the study of Iyasere et al. (17).

In this study, we made use of tests that have been validated in chickens as a measure of fear. The open field test was not undertaken because the response of animals in this test is a combination of two motivations: fear and the need for social reinstatement (37). We adopted the TI test as a measure of the level of fear in the current study because TI is an anti-predator freezing response (feigning death) in which prey species adopt a relatively immobile state that can last from seconds to hours after the physical restraint has ceased (38–40). The TI can function to reduce the perceived need of the predator to further subdue the prey, thereby increasing opportunities for the prey to escape and survive (40–42). A predator model is an established method to score individual variations in fear (37).

The YRE and FLE showed consistency in the number of attempts to induce TI and the duration of TI over the three testing time points. This implies a stable fear response over the first three weeks of life, which happens to be the most critical point contributing to their survivability. In addition, testing the chicks once a week for three weeks did not induce any form of habituation. Studies have reported that chicks get accustomed to TI, showing reduced susceptibility and duration to TI when they are subjected to repeated daily testing (43, 44).

From the behavioral responses of the chicks to the simulated predator test, we observed that a higher percentage of the chicks of both ecotypes seem to belong to the "fearful" category. This suggests that the chicks perceived the simulated

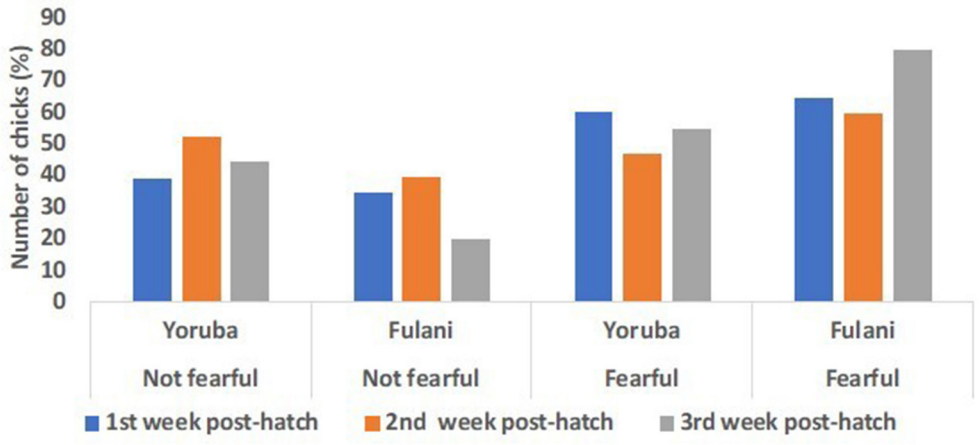

FIGURE 11
Predator fear score responses in two ecotypes (1 = Yoruba and 2 = Fulani) of Nigerian indigenous chicks at weeks 1–3 (i.e., day 8 (blue bars), day 15 (orange bars), and day 22 (gray bars) post-hatch).

predator as a potential one and adopted behaviors such as freezing, crouching, or running to escape from it. From an evolutionary point of view, these behaviors may enhance fitness and survival in the wild (37). From the TI and simulated predator tests used in the current study, the YRE chicks were more fearful, having a longer duration of TI on day 7 PTH, and they easily entered TI on day 14 PTH. Despite this interesting finding in the differences between the ecotypes, it is worth mentioning that the interpretations of fear responses and their implications on welfare seem to be context-dependent. The display of a high level of fear in birds housed in an intensive system may be considered counterproductive as this could result in piling and smothering leading to injury and even death. However, in the natural environment (wild) or for birds that are considered for free range systems, the birds need to show appropriate behavioral responses, which endows them with better fitness and survivability.

Based on the variability in the fear responses of the chicks of the two ecotypes, we can suggest that the two ecotypes can be considered as chicken ecotypes suitable for different housing systems; the YRE for an outdoor/free range system because of their ability to escape from predators by displaying a high level of fear; and the FLE for an indoor production system. However, further studies would be required to validate this, as Lindholm et al. (45) reported that longer tonic immobility observed in the slow-growing broiler strain (Rowan Ranger) did not affect their use of the range. The level of fear appears to be influenced by body weight. The increased level of fear in the YRE could also be related to the lower body weight compared to the FLE chicks. Further studies on the influence of age and body weight on the level of fear experienced by these two ecotypes will be needed.

Conclusion

This study observed some influence of ecotype on maternal behaviors of Nigerian indigenous hens during brooding and the level of fear of their chicks. The YRE hens spent more time sitting on the eggs. The FLE chicks had a lower level of fear in the TI test but showed a higher fear response to simulated predator attack, which is needed in case the bird is exposed to a real-life predator. Results from this study show that the FLE hens can be recommended as "ecotype with good welfare" with better feed conversion and produce more meat and eggs to meet the nutritional requirements of man and have economic benefits to the rural poor farmers. The welfare of the chicks in terms of fear and behavioral responses to escape from predators could be a potential criterion that can be used to determine the best housing system for the ecotypes. The YRE ecotype showing higher predator escape behavior may be considered for free-range housing production because this behavior can enhance their survivability in the natural environment when faced with real-time predators.

We therefore recommend an improvement in both ecotypes using the appropriate breeding programs that would improve the productivity (feed conversion, meat and egg) of FLE in an intensive management system, and broodiness as well as survivability of YRE under an extensive system.

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.

Ethics statement

All procedures of this study were based on guidelines of the Animal Care and Use Committee of the Federal University of Agriculture, Abeokuta, Ogun State Nigeria.

Author contributions

VO, OI, SD, and JD were involved in the conceptualization, design, implementation, and manuscript writing. FF, POdeta, SO, POdetu, and TA were involved in fieldwork, data collection, and extraction of behavioral data from the video. All authors contributed to the article and approved the submitted version.

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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

References

1. Manyelo TG, Selaledi L, Hassan ZM, Mabelebele M. Local chicken breeds of Africa: their description, uses and conservation methods. *Animals.* (2020) 10:2257. doi: 10.3390/ani10122257

2. Yakubu A, Ogah DM, Barde RE. Productivity and egg quality characteristics of free range naked neck and normal feathered Nigerian indigenous chickens. *Int J Poult Sci.* (2008) 7:579–85. doi: 10.3923/ijps.2008.579.585

3. Lasagna E, Ceccobelli S, Cardinali I, Perini F, Bhadra U, Thangaraj K, et al. Mitochondrial diversity of Yoruba and Fulani chickens: a biodiversity reservoir in Nigeria. *Poult Sci.* (2020) 99:2852–60. doi: 10.1016/j.psj.2019.12.066

4. Islam MA, Nishibori M. Indigenous naked neck chicken: a valuable genetic resource for Bangladesh. *World's Poultry Sci J.* (2009) 65:125–38. doi: 10.1017/S0043933909000105

5. Ogbu CC, Tule JJ, Nwosu CC. Effect of genotype and feeding plan on growth and laying parameters of Nigerian indigenous chickens. *Global J Biol Agric Health Sci.* (2015) 4:251–6.

6. FAO. *In Vivo Conservation of Animal Genetic Resources. FAO Animal Production and Health Guidelines.* (2013). Rome, Italy. Available online at: http://www.fao.org/3/i3327e/i3327e00.htm (accessed December 30, 2019).

7. Yakubu A, Kuje D, Okpeku M. Principal components as measures of size and shape in Nigerian indigenous chickens. *Thai J Agric Sci.* (2009) 42:167–76.

8. Olawunmi OO, Salako AE, Afuwape AA. Morphometric differentiation and asessment of function of the Fulani and Yoruba ecotype indigenous chickens of Nigeria. *Int. J. Morphol.* (2008) 26:975–80. doi: 10.4067/S0717-95022008000400032

9. Sola-Ojo FE, Ayorinde KL. The Fulani ecotype chicken: Growth and Feed utilization potentials. *World J Appl Sci Technol.* (2009) 1:37–45.

10. Jesuyon OM. Salako AE. Variability and predictability of productive and body traits of Fulani ecotype chicken. *Afr J Agric Res.* (2013) 8:6178–84.

11. Momoh OM, Tule JJ, Nwosu CC. Backward integration in natural incubation of local chickens using the basket system. In: *Proceedings of the 29th annual conference of the Nigerian Society for Animal Production 21st - 25th March.* (2004). p. 36–9.

12. Osaiyuwu OH, Salako AH, Adurogbangban O. Body dimensions of Fulani and Yoruba ecotype chickens under intensive systems of management. *J Agric For Soc Sci.* (2009) 7:14–7. doi: 10.4314/joafss.v7i2.64358

13. Ige AO. Quantitative differentiation of two population of indigenous chickens in a derived savannah zone of Nigeria using morphometric traits. *Int J Res Stud Biosci.* (2014) 2:1–6.

14. Sola-Ojo FE, Ayorinde KL, Jatto OM, Toye AA. Comparative studies of two Nigerian ecotypes chicken kept in battery cages for laying performance and egg quality traits. *Asian J Agric Rural Dev.* (2013) 3:38–45. Retrieved from: https://archive.aessweb.com/index.php/5005/article/view/650

15. Ajayi FO. Nigerian indigenous chicken: A valuable genetic resource for meat and egg production. *Asian J Poultry Sci.* (2010) 4:164–72. doi: 10.3923/ajpsaj.2010.164.172

16. Momoh OM, Ehobu NG, Nwosu CC. Egg production of two local chicken ecotypes under improved management. In: *proceedings of the 32nd Annual Conference of Nigeria Society for Animal Production Calabar.* (2007) 149–51.

17. Iyasere OS, Ajayi OD, Alade SO, Akinbode VO. Behaviour, physiology and body mass of Nigerian indigenous hens during brooding. *Agric Tropica et Subtropica.* (2019) 52:43–7. doi: 10.2478/ats-2019-0005

18. Iyasere OS, Famosaya OO, Oyeniran VJ, Durosaro SO, Majekodunmi BC. Which is more stressful: physical or visual separation of hen from chicks? In: *Proceedings of the 54th Congress of the ISAE 2nd August – 6th August.* (2021). p. 10.

19. Farm Animal Welfare Council. FAWC updates the five freedoms. *Vet Rec.* (1992) 131:357. doi: 10.1016/B978-0-12-394586-0.00009-3

20. Muir W, Cheng HW. Genetic influences on the behaviour of chickens associated with welfare and productivity. In: *Genetics and the Behavior of Domestic Animals.* (2014). p. 317–59.

21. Pittet F, Coignard M, Houdelier C, Richard-Yris MA, Lumineau S. Effects of maternal experience on fearfulness and maternal behaviour in a precocial bird. *Anim Behav.* (2013) 85:797–805. doi: 10.1016/j.anbehav.2013.01.026

22. Rodenburg TB, Van Hierden YM, Buitenhuis AJ, Riedstra B, Koene P, Korte SM, et al. Feather pecking in laying hens: new insights and directions for research? *Appl Anim Behav Sci.* (2004) 86:291–8. doi: 10.1016/j.applanim.2004.02.007

23. Rodenburg TB, Bolhuis JE, Koopmanschap RE, Ellen ED, Decuypere E. Maternal care and selection for low mortality affect post-stress corticosterone and peripheral serotonin in laying hens. *Physiol Behav.* (2009) 98:519–23. doi: 10.1016/j.physbeh.2009.08.006

24. Wauters AM, Richard-Yris MA, Talec N. Maternal influences on feeding and general activity in domestic chicks. *Ethology.* (2002) 108:529–40. doi: 10.1046/j.1439-0310.2002.00793.x

25. De Margerie E, Peris A, Pittet F, Houdelier C, Lumineau S, Richard-Yris MA. Effect of mothering on the spatial exploratory behavior of quail chicks. *Dev Psychobiol.* (2013) 55:256–64. doi: 10.1002/dev.21019

26. Riber AB, Wichman A, Braastad BO, Forkman B. Effects of broody hens on perch use, ground pecking, feather pecking and cannibalism in domestic fowl (*Gallus gallus domesticus*). *Appl Anim Behav Sci.* (2007) 106:39–51. doi: 10.1016/j.applanim.2006.07.012

27. Hewlett SE, Nordquist RE. Effects of maternal care during rearing in White Leghorn and Brown Nick layer hens on cognition, sociality and fear. *Animals.* (2019) 9:454. doi: 10.3390/ani9070454

28. Hewlett SE, Zeinstra EC, van Eerdenburg FJCM, Rodenburg TB, van Kooten PJS, van der Staay FJ, et al. Hypothalamic vasotocin and tyrosine hydroxylase levels following maternal care and selection for low mortality in laying hens. *BMC Vet Res.* (2014) 10:167. doi: 10.1186/1746-6148-10-167

29. Jones RB, Faure JM. Tonic immobility ("righting time") in laying hens housed in cages and pens. *Appl Anim Ethol.* (1981) 7:369–72. doi: 10.1016/0304-3762(81)90063-8

30. Rodenburg TB, Uitdehaag KA, Ellen ED, Komen J. The effects of selection on low mortality and brooding by a mother hen on open-field response, feather pecking and cannibalism in laying hens. *Anim Welfare.* (2009) 18:427–32.

31. Ohkubo T, Tanaka M, Nakashima K, Talbot RT, Sharp PJ. Prolactin receptor gene expression in the brain and peripheral tissues in broody and nonbroody breeds of domestic hen. *Gen Comp Endocrinol.* (1998) 109:60–8. doi: 10.1006/gcen.1997.7008

32. Romanov MN, Talbot RT, Wilson PW, Sharp PJ. Genetic control of incubation behavior in the domestic hen. *Poult Sci.* (2002) 81:928–31. doi: 10.1093/ps/81.7.928

33. Tona K, Onagbesan O, De Ketelaere B, Decuypere E, Bruggeman V. Effects of turning duration during incubation on corticosterone and thyroid hormone levels, gas pressures in air cell, chick quality, and juvenile growth. *Poult Sci.* (2003) 82:1974–9. doi: 10.1093/ps/82.12.1974

34. Hepp GR, DuRant SE, Hopkins WA. Influence of incubation temperature on offspring phenotype and fitness in birds. In: *Nests, Eggs, and Incubation (Deeming DC, Reynolds SJ)*. Oxford, UK: Oxford University Press (2015). p. 171–78.

35. Breitenbach RP, Meyer RK. Effect of incubation and brooding on fat, viscera weights and body weight of the hen pheasant (*Phasianus colchicus. Poult Sci.* (1959) 38:1014–26. doi: 10.3382/ps.0381014

36. Azharul IM, Ranvig H, Howlider MAR. Incubating capacity of broody hens and chick performance in Bangladesh. *Livestock Res Rural Dev.* (2005) 17:1–10. Available online at: http://www.cipav.org.co/lrrd/lrrd17/2/azha17019.htm

37. Forkman B, Boissy A. Meunier- Salaün, M-C, Canali E, Jones RB. A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. *Physiol Behav.* (2007) 92:340–74. doi: 10.1016/j.physbeh.2007.03.016

38. Gallup GG. Animal hypnosis: factual status of a fictional concept. *Psychol Bull.* (1974) 81:836. doi: 10.1037/h0037227

39. Rogers SM, Simpson SJ. Thanatosis. *Curr Biol.* (2014) 24:R1031–3. doi: 10.1016/j.cub.2014.08.051

40. Humphreys RK, Ruxton GD, A. review of thanatosis (death feigning) as an anti-predator behaviour. *Behav Ecol Sociobiol.* (2018) 72:1–6. doi: 10.1007/s00265-017-2436-8

41. Miyatake T, Katayama K, Takeda Y, Nakashima A, Sugita A, Mizumoto M. Is death–feigning adaptive? Heritable variation in fitness difference of death–feigning behavior. *Proc Royal Soc Lond Ser B: Biol Sci.* (2004) 271:2293–6. doi: 10.1098/rspb.2004.2858

42. Cassill DL, Vo K, Becker B. Young fire ant workers feign death and survive aggressive neighbors. *Naturwissenschaften.* (2008) 95:617–24. doi: 10.1007/s00114-008-0362-3

43. Nash RF, Gallup GG. Habituation and tonic immobility in domestic chickens. *J Comp Physiol Psychol.* (1976) 90:870–6. doi: 10.1037/h0077265

44. Nash RF. Habituation and tonic immobility in chickens: strain comparisons. *Psychol Rec.* (1978) 28:109–14. doi: 10.1007/BF03394514

45. Lindholm C, Karlsson L, Johansson A, Latimiras J. Higher fear of predators does not decrease outdoor range use in free-range Rowan Ranger broiler chickens. *Acta Agriculturae Scandinavica, Section A- Animal Science.* (2016) 66:231–8. doi: 10.1080/09064702.2017.1337214

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Environmental enrichment improves the growth rate, behavioral and physiological response of juveniles of *Clarias gariepinus* under laboratory conditions

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Environmental enrichment (EE) improves the growth rate and welfare of some cultured fishes. However, most cultured fish species are raised in non-enriched housing conditions. *Clarias gariepinus* is an important commercial fish species, but little is known about the effect of EE on their welfare. This study examined the effect of different EE on the survival rate (SR), growth [mean weight gain (MWG), specific growth rate (SGR) and feed conversion ratio (FCR)], behavioral (feed response, aggressive acts and shoaling time) and physiological responses (blood glucose) of *C. gariepinus*. *One hundred* and twenty juveniles of *C. gariepinus* (31.65 ± 0.69 g) were randomly allocated at 10 fish/tank and subjected to either Plant Enriched (PE), Substratum Enriched (SE), Plant and Substratum Enriched (PSE) and Non-Enriched (NE) tanks in triplicates for 56-days. Behavioral acts were observed for 10 min twice daily, and glucose level in blood samples was evaluated. Data were checked for normality using the Shapiro-Wilk test before being analyzed with the Kruskal-Wallis test. SR and MWG were significantly higher in *Clarias gariepinus* exposed to SE, with no significant differences among PE, PSE and NE treatments. There was no significant difference between the SGR of PSE and NE. FCR was similar between treatments. The highest condition factor (k) was recorded in SE tanks. Duration of feed response was shorter in SE, but there was no significant difference between the feed response of *C. gariepinus* exposed to PE and PSE. *C. gariepinus* exposed to PE, SE and PSE displayed a similar frequency of aggressive acts. African catfish reared in NE (barren) tanks had the least duration of shoaling period. The experiment consistently found the highest and least glucose values in PSE and SE. In conclusion, environmentally enriched housing

tanks with SE resulted in the best MWG with a reduced level of aggression in *C. gariepinus* under laboratory conditions. Thus, EE might be applicable to boost fish productivity on a commercial scale.

KEYWORDS

aggressive, African catfish, enrichment, fish welfare, stress in fish

Introduction

The aquaculture sector makes a tremendous global contribution to the development of a nation in terms of provision of employment, fish food security, nutritional diet and a trade commodity for export (1). The aquaculture industry has been the fastest-growing global agro-industrial sector in the last four decades (2, 3). FAO Organization (4) and Franks and Ewell (5) reported a total of 82.12 million metric tons of farmed aquatic animals, which constitute around 250–408 billion fish species for the rising global human population. Furthermore, the aquaculture industry offers a great potential for boosting fish production at a rate that can outpace the rising domestic demand if the welfare of the cultured fish species is improved (3, 6, 7). However, most conventional rearing environments for fish culture are mostly barren. They lack a physical form of improvement or enrichments that could aid natural behavior in cultured fish to promote an optimum growth rate and welfare (8, 9). Interestingly, improving the rearing environment of cultured fish species is welfare friendly (6, 10, 11) and can serve as a growth booster (9, 12); thus, applying environmental enrichment could improve the production rate of *Clarias gariepinus* species for sustainability.

Clarias gariepinus (African catfish) is Africa's most popularly cultured finfish by Aquaculturists (2, 13). The fish is highly preferred for its ease of culture, general acceptability and high economic value (14). Furthermore, fish farmers mostly prefer the species due to its resistance to diseases, hardiness and fast growth rate (15). African catfish can tolerate a wide range of freshwater habitats and can still survive for weeks when they burrow into the sediment and mud of ponds. Consequently, *C. gariepinus* (African catfish) is well-studied in terms of nutrition (16, 17), feeding behavior (18, 19), management and reproduction techniques (20) and welfare (21–23), among others. In addition, Hossain and Beveridge (24) studied the effect of light and shelter on the growth and survival of *C. gariepinus,* Schram et al. (25) enriched the diet of *C. gariepinus* with functional selenium, Arechavala-Lopez et al. (26) reviewed the effect of environmental enrichment on cultured fish species and (22) assessed the effect of chronic stressor on welfare indicators of *C. gariepinus*. However, to the best of our knowledge, there is a paucity of information on the effect of environmental enrichment on growth indices, behavioral and the general wellbeing of this important tropical fish species, which calls for urgent attention to improve fish production efficiency and welfare.

Animal welfare can be described as the feelings experienced by animals, i.e., the presence of positive feelings or pleasure and the absence of strong negative feelings or suffering in the rearing environment of the animal (27, 28). Interestingly, animal welfare protections have been established for a variety of farmed species in developed countries (29, 30), yet the concept of fish welfare is gaining increasing public interest in developing countries. In most cases, fish are often categorized as aquatic animals, and their welfare is most often ignored in animal welfare decision-making policies. However, Sneddon et al. (31) and Brown (32) described fish as sentient beings that can experience good or bad feelings, pain or emotional states. In addition, Mason and Lavery (33) reviewed the uncertainty of the sentience nature of fish and opined that it is imperative to protect the welfare of fish and treat them as sentient animals. Consequently, the welfare of fish species must be given utmost attention to develop the aquaculture sector for sustainability. In the same vein, the utilization of different forms of environmental enrichment to improve the welfare of cultured fishes has remained an important global issue that is mostly being pursued by researchers, animal rights organizations and many producers to improve the productivity and welfare of fish species toward sustainability (26, 34–37). However, until now, environmental enrichment has not been applied to improve the welfare of tropical aquatic animals such as African catfish.

Environmental enrichment involves the conscious addition of environmental complexities to the rearing enclosures of fish species to mimic the natural habitat and improves the welfare of the farmed fish species (38–40). This complexity could be in the form of social, feeding, cognitive, structural or physical forms of enrichment (26). The physical form of enrichment involves the provision of structures like plants, sediments, stones, kelps, sand, gravels, artificial objects etc. in the rearing environment of captive fish to create a sensory and motor simulation that suites the behavioral and physiological needs of the fish (6, 41). Moreover, it offers the opportunity to use natural materials (plants, substratum) found within the fish species' habitat to improve their welfare without necessarily increasing the cost of production. *Eichhornia crassipes* (water hyacinth) are prevalent at the surface of many tropical and

sub-tropical aquatic environments (42). The plant is available with a high proliferation rate and capacity to absorb nutrients in the tropical region (43). For instance, improved physico-chemical parameters of rearing water and higher growth rate were found in *Clarias gariepinus* reared in fish enclosures enriched with water hyacinth (43). In addition, Brunet et al. (41) found a positive welfare effect of the nature-based physical form of enrichment on farmed rainbow trouts. A reduced level of aggressive behavior was reported in two territorial fishes and *Tilapia rendalli* exposed to physical and structural enrichments (44, 45). Thus, the utilization of physical enrichment materials found in the natural habitat of *Clarias gariepinus,* which pose little or no financial implication, could be applied to enrich the rearing enclosures of this fish species for improved biological functioning and wellbeing.

Duncan (27) opined that the measurements of impaired biological functioning related to decreased health and increased physiological stress response could provide evidence that the welfare of an animal is compromised. Moreover, cortisol and glucose are the most commonly used indicators of the physiological response of teleost to stress (46, 47). Nevertheless, Broom (48) stated that cortisol provides no evidence of poor welfare because it has roles in positive and negative situations, which makes it erroneous to interpret its value as an indicator of poor welfare. However, variations in blood glucose levels are a primary stress response. It is a reliable biomarker to assess the hypothalamic-pituitary-adrenal (HPA) axis that indicates the condition and additional welfare benefits (49). The presence of stressors in the rearing enclosures of fish species considerably impacts their physiology, welfare and productivity. David et al. (50) reported using blood glucose and glycogen as indicators of stress response in freshwater fish species. Similarly, Endo and Wu (51) reviewed the use of blood glucose and cortisol as a good measure of assessing stress and fish welfare. Malini et al. (52) reported increased blood glucose as a physiological response in fishes exposed to environmental disturbance. In addition, Hossain and Beveridge (24) categorized the survival and growth rate trend in a fish rearing system as a crucial determinant of production success and an unambiguous indicator of animal wellbeing in a confined environment.

Behavioral indicators represent a generally non-invasive and early warning system of poor conditions in an aquatic environment (53). These behavioral tools include alteration in feed response, shoaling, aggression acts, swimming behavior etc., within the rearing enclosures of the fish (6). The rate of feed response in cultured fish species indicates the growth and production success of the farmer in the aquaculture sector (18, 54). Martins et al. (55) reported the relevance of shoaling behavior as a defensive behavior against predators and a good indicator of positive welfare in farmed fish species. In addition, Martins et al. (55) and Salvanes et al. (56) categorized changes in foraging behavior, aggression and group swimming of farmed fish species as an indication of acute and chronic stressors within the rearing environment. Moreover, the role of EE on behavioral traits of cultured fish species has been extensively documented (8, 9, 12, 38, 45). For instance, increased shoaling rate of cultured fish species reduced physical attacks, fights, food acquisition, and successful foraging behavior in zebrafish (56–59). Rosburg et al. (60) and Whiteet al. (61) verified the positive effect of environmental enrichment on the growth of chinook salmon, brown and rainbow trout. At the same time, Arechavala-Lopez et al. (39) and Zhang et al. (12) reported the positive influence of environmental enrichment on the growth, behavioral, physiological and welfare of *Sparus aurata* and *Sebastes schlegelii* in a laboratory environment.

However, there is a scarcity of information on studies related to the cumulative effect of environmental enrichment on *C. gariepinus* and its potential for application in the commercial production of this species. In this study, we hypothesized that all the provided forms of environmental enrichment during the 56-day culture period would improve the general well-being of *Clarias gariepinus.* Thus, the hypothesis of this study predicts that the provided forms of environmental enrichment would boost the survival rate and growth indices of the fish species while reducing the level of aggression and the blood glucose of the juveniles of the *Clarias gariepinus* under laboratory conditions.

Materials and methods

Study location

The research was carried out at the FUNAAB fish laboratory located between latitude $7^0$10'N and longitude $3^0$2'E.

Experimental fish and acclimatization procedure

One hundred and eighty juveniles of *Clarias gariepinus* of 9-week-old were purchased from a private fish farm and transported in oxygen-filled polythene bags at 0700 h to the study site. The fish were acclimatized for 14 days in a rectangular fiber tank (6 x 4 x 3 m); they were fed twice daily (0900 h and 1,700 h) with Coppens feed (3 mm, Crude Protein = 45% and crude lipid = 12%, 4,300 kcal of digestible energy $kg^{-}1$) at 3% body weight (16, 54).

Experimental design and procedure

A total of 120 juveniles of active *C. gariepinus* with an average weight of 31.65 ± 0.69 g and a standard length of 11.2 ± 0.13 cm were selected from the purchased 180 catfish in the acclimatization tank. The fish were randomly stocked in

12 plastic tanks (1.7 × 1.2 × 1.0 m) of four treatments at 10 fish per tank in triplicates, representing an average weight of African catfish stocked per cubic meter (62). Each treatment was randomly exposed to plant enriched (PE), substratum enriched (SE), plant and substratum enriched (PSE), and barren/non-enriched (NE) tanks for a culture period of 56-days (11, 44). Each PE tank was filled with 10–12 stands of *Eichhornia crassipes,* popularly called water hyacinth; each stand contains 4–5 leaves with an average height of 15–20 cm above the water surface. The floating plants were collected from the outdoor fish enclosures located 10 m from the study site. The plants were washed with borehole water to get rid of snails and other likely pathogens; it was washed for 2 min under de-ionized water before placing them evenly at the surface of the plastic tanks (9). The SE tanks were filled with washed and sterilized fine sand substratum (grain size of 0.5–2.00 mm) to a depth of 1.5 cm in the culture tanks (8); PSE tanks were mixed at a ratio of one part of water hyacinth plant to one part of the fine sand substratum. The NE tanks were barren and plain, without any form of enrichment added to the tanks. The forms of enrichment used in this study are similar to what is obtainable in natural aquatic environments without any chemical interaction, which conforms with the specifications of Zhang et al. (12). Each tank was filled with water to two-thirds of its capacity with a flow-through system at a rate of 2.4 lhr^{-1}. A weekly general tank cleaning and partial water exchange (9) were carried out throughout the study. The sides of the tanks were covered with opaque polythene material to reduce disturbance and interference during the study. All the 12 tanks were kept in the same laboratory room and exposed to the same photoperiod regime of 12L:12D. The water quality parameters were monitored with a multiparameter water probe (HANNA HI 98107 and HI 9143), and the mean values recorded during the experimental period for dissolved oxygen, temperature and pH were 6.5 ± 0.08 mg/l, 28.7 ± 1.0^0C and 6.70 ± 0.51, respectively.

Feeding pattern and growth indicators

The fish in each treatment and tank were fed with Coppens feed (3 mm, Crude Protein = 45% and crude lipid = 12%, 4 300 kcal of digestible energy $kg^{-}1$) at a feeding rate of 3% per body weight. They were fed twice a day at 0900 h and 1,700 h (general feeding time) using the broadcasting method to ensure uniform access to feed by all the stocked fish. All uneaten feed (if any) was removed 30 min after feeding to prevent an alteration in the water quality in the culture tanks. Fish were weighed weekly to the nearest 0.01 g using Metler weighing balance (Model: 1,106) for an adjustment in the quantity of feed offered, while the standard length of fish was measured with a measuring board to the nearest 1.0 cm.

The initial body weight and weekly weight gain of the stocked *C. gariepinus* were recorded appropriately throughout the experimental period. The growth indicators (mean weight gain (MWG), specific growth rate (SGR) and feed conversion ratio (FCR) were evaluated for each of the treatments as follows:

$$Weight\ gain\ (g) = Final\ weight - initial\ weight. \quad (1)$$

$$Specific\ Growth\ Rate\ (g/day) = \left([\ln(FW) - ln(IW)]/t\right)^{*} 100 \quad (2)$$

Where ln = Natural logarithm
FW = Final weight
IW = Initial weight
t = Duration of the experiment (in days) (63).

$$Feed\ Conversion\ Ratio = Feed\ intake\ (g)\ /Bodyweight\ gain\ (g) \quad (3)$$

(16).

Survival rate

The survival rate (SR) of all the experimental African catfish was estimated using the equation illustrated below:

$$Survival\ rate\ = [(INF\ -\ FNF)/INF]^{*}100 \quad (4)$$

Where INF = Initial number of fish stocked
FNF = Final number of fish stocked (16).

Condition factor (k)

The condition factor (k) of the fish species was calculated to state their general wellbeing using Fulton's equation (64):

Condition factor (k)
$k = \frac{100W}{L3}$.
Where k = Condition factor
W = Body weight(g)
L = Standard length (cm)

Quantification of behavioral acts

Juveniles of *C. gariepinus* were observed at 08:00 h and 16:00 h, twice a week fortnightly, for 10 min per scan sampling using a focal sampling technique. The tanks were completely randomized for an appraisal at every observation time to eliminate bias. Each tank and treatment was observed for 120 min throughout the study period by two observers (with a timekeeper per observer). The sidewalls of the rearing tanks

TABLE 1 Ethogram of the measured behavioral variables.

Behavioral traits	Description
Feed response	Duration (in minutes) of time used by the fish to consume their given ration of feed
Aggressive acts	Frequency of instances of chasing that leads to contact between the mouth and body of a fish to inflict a mark or injury
Shoaling behavior	Duration (in seconds) of swimming together in clusters (six fish or more at $\leq$ 5 cm apart) at the lower one-third of the tank

were covered with black polythene materials to prevent human disturbance during behavioral assessment (19, 54). The feeding response of the *C. gariepinus* in each treatment was assessed with a stopwatch. In addition, the frequency of aggressive acts displayed by the fish within the 10 min of observation time was counted and recorded in each tank per treatment. Furthermore, the duration of shoaling at the bottom of the tank as described by Miller and Gerlai (59) during the 10 min of observation by each treatment was recorded appropriately. The description of the behavioral traits measured in the study is given in Table 1.

Blood sampling and measurement of blood glucose

Blood samples were collected fortnightly during the 56-day culture period to determine the physiological effect of the different forms of environmental enrichment on the stocked *C. gariepinus*. The blood samples were collected between 0700 and 0900 h. Sampled fish species ($n = 3$) per treatment were netted from the experimental tanks and anesthetized with MS222 in a 20 litres bucket of water; blood samples were collected at the caudal vein using a 2.5 ml heparinized syringe with 22G x 1½" according to the method of Di Marco et al. (65). Collected blood was gently pushed into a sterilized microfuge tube containing anticoagulant (20 mM EDTA). The whole blood withdrawal process took < 5 min per fish to prevent discomfort. The samples were analyzed for blood glucose at the central Biotechnology Laboratory of the Federal University of Agriculture Abeokuta using the spectrophotometric method (47).

Statistical analysis

All data obtained during the experiment were analyzed using the routines of IBM SPSS statistical packages (Version 23). The data were tested for normality using Shapiro-Wilk's test, while the homogeneity of data was tested using Levene's test. All the obtained data were not normally distributed and did not meet the assumption of ANOVA on normality and homogeneity even after transformation. The data were subjected to Kruskal-Wallis, a non-parametric test. Significant differences were reported at an alpha level of 0.05.

Results

Survival rate

At the end of the 8 weeks study, there was a significant ($\chi^2 = 77.31$, df = 3, $p = 0.01$) difference in the survival rates of juveniles of *C. gariepinus* exposed to the different levels of environmental enrichment. The highest survival rate (83.4%) was recorded in SE, and there was no significant difference in the survival rates recorded in PE, PSE and NE throughout the study period (Figure 1).

Growth indicators and condition factors of juveniles of *Clarias gariepinus*

No significant ($\chi^2 = 31.75$, df = 3, $p = 0.14$) difference was observed between the initial body weight of *C. gariepinus* exposed to the different forms of environmental enrichment (Table 2). However, at the end of the culture period, a significant difference was observed in the final weight ($\chi^2 = 90.51$, df = 3, $P = 0.02$) and mean weight gain ($\chi^2 = 58.87$, df = 3, $P = 0.04$) of the cultured juveniles of *C. gariepinus* (Table 2). Fish reared with a substratum (SE) form of environmental enrichment had the highest mean weight gain (MWG) compared to the other forms of enrichment. The final body weight and MWG found in PE and NE were similar. In addition, the EEs had a significant ($\chi^2 = 2.11$, df = 3, $p = 0.01$) difference in the SGR of the cultured fish, but there was no significant difference between the SGR obtained at PSE and NE at the end of the culture period. Other estimated growth indices, such as FCR, were similar ($\chi^2 = 1.04$, df = 3, $p = 0.10$) between treatments after the 56 days of exposure (Table 2). Also, the condition factor of the *C. gariepinus* was similar at the beginning (week 1) and end (week 8) of the culture period. Still, a higher k-value was recorded in the fish exposed to SE enrichment (Figure 2).

Behavioral traits of *C. gariepinus*

A significant difference was observed in the behavioral acts displayed by the juveniles of *C. gariepinus* exposed to the different forms of environmental enrichment throughout the study period. The fish in SE tanks took a shorter time to consume

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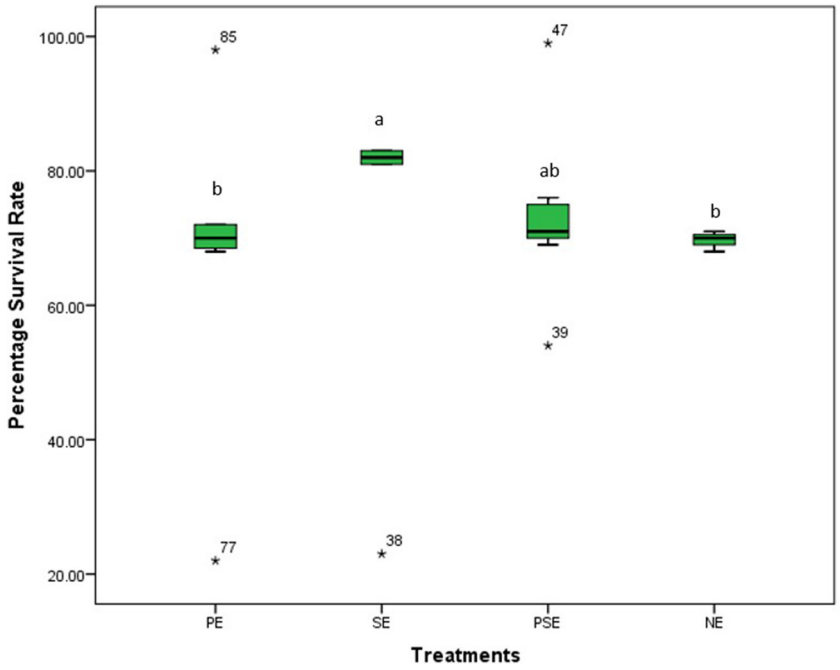

FIGURE 1
The survival rates of *Clarias gariepinus* exposed to plant enriched (PE), substratum enriched (SE), plant and substratum enriched (PSE) and non-enriched (NE) culture tanks. A significant difference between treatments was indicated with different letters at $p < 0.05$.

TABLE 2 The growth parameters of *C. gariepinus* exposed to different forms of environmental enrichment under laboratory conditions.

Growth indices	Plant enriched (PE.)	Substratum enriched (SE.)	Plant and substratum enriched (PSE)	Non-enriched (NE.)
Initial body weight (g/fish)	31.64 ± 0.64^{a}	31.63 ± 0.62^{a}	31.64 ± 0.64^{a}	31.68 ± 0.68^{a}
Final body weight (g/fish)	88.83 ± 0.94^{c}	93.52 ± 1.03^{a}	91.37 ± 0.97^{b}	88.33 ± 0.91^{c}
Mean weight gain (g/fish)	57.19 ± 0.51^{c}	61.89 ± 0.59^{a}	59.73 ± 0.55^{b}	56.65 ± 0.48^{c}
SGR (%/day)	1.96 ± 0.11^{c}	2.25 ± 0.17^{a}	2.13 ± 0.12^{b}	2.11 ± 0.09^{b}
FCR	1.01 ± 0.06^{ab}	1.09 ± 0.09^{a}	1.06 ± 0.05^{b}	1.01 ± 0.06^{ab}

[abc]Mean values with different superscripts within a row are significantly ($P < 0.05$) different between treatments.
SGR, Specific Growth Rate; FCR, Feed Conversion Ratio.

their diet. There was no significant difference ($\chi^2 = 6.58$, df = 3, $P = 0.09$) between the feed response of *C. gariepinus* exposed to PE and PSE forms of enrichment (Figure 3). In addition, the time of feeding during the day (morning and evening) had no significant effect on the feed response of the fish exposed to the different forms of environmental enrichment (Figure 4). Throughout the culture period, similar levels of aggressiveness were displayed by the juveniles of *C. gariepinus* exposed to PE ($\chi^2 = 23.22$, df = 3, $P = 0.03$), SE ($\chi^2 = 19.93$, df = 3, $P = 0.04$), and PSE ($\chi^2 = 21.04$, df = 3, $P = 0.03$) forms of enrichments. In addition, the highest ($\chi^2 = 31.61$, df = 3, $P = 0.02$) number of aggressive acts were displayed by fish reared in NE tanks compared to the other *C. gariepinus* cultured in other forms of EE treatments throughout the experimental period (Figure 5).

Also, the different EE treatments did not affect ($\chi^2 = 391.42$, df = 3, $P = 0.10$) the duration of shoaling displayed by *C. gariepinus*. There were similarities in the duration of shoaling behavior displayed by fish reared in PE ($\chi^2 = 433.17$, df = 3,

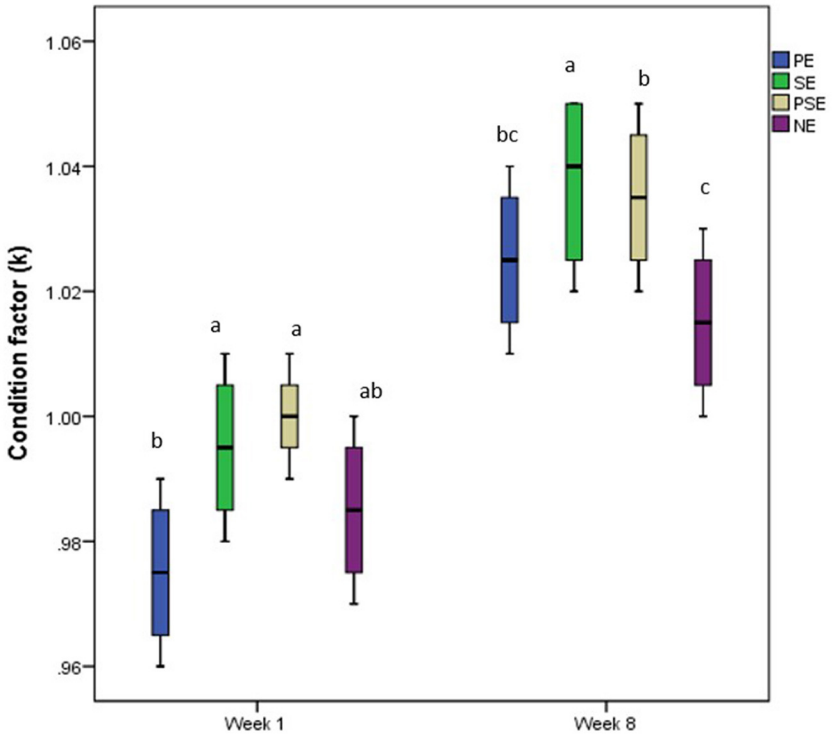

FIGURE 2
Change in condition factor (k) of juveniles of *Clarias gariepinus* exposed to different forms of environmental enrichment (PE, Plant enriched; SE, substratum enriched; PSE, plant and substratum enriched; NE, non-enriched) at week one and eight of the experimental period.

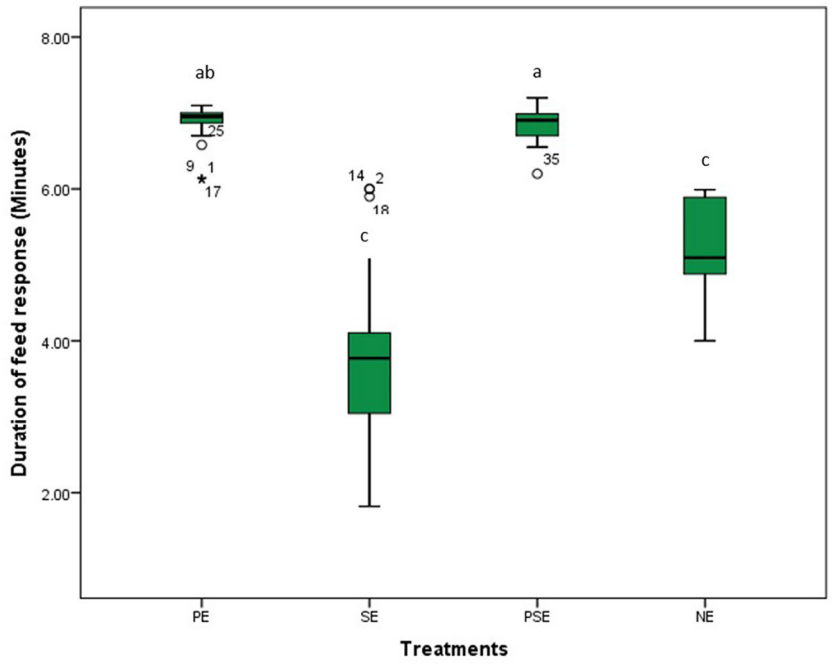

FIGURE 3
The effect of environmental enrichments on the feed response of juveniles of *Clarias gariepinus* under laboratory conditions. [abcd] Means of the duration of feed response differ ($p < 0.05$) between treatments.

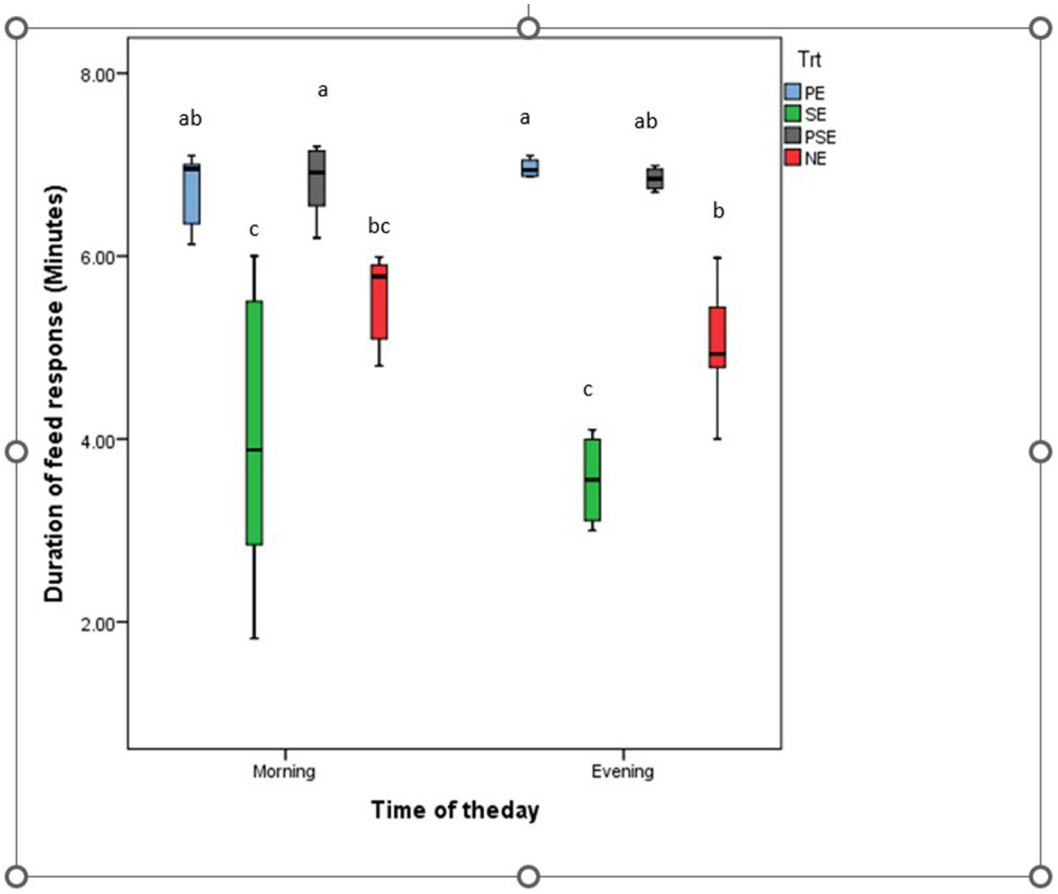

FIGURE 4
Average duration of feed response in juveniles of *Clarias gariepinus* exposed to different forms of environmental enrichment (PE, Plant enriched; SE, substratum enriched; PSE, plant and substratum enriched; NE, non-enriched) at two different times (morning and evening) of the day. Significant differences between treatments at each experimental period are marked with different superscripts at $p < 0.05$.

$P = 0.04$), PSE ($\chi^2 = 441.09$, df = 3, $P = 0.02$) and SE ($\chi^2 = 429.89$, df = 3, $P = 0.04$) tanks. Besides, the least duration of shoaling within the period of observation was displayed by *C. gariepinus* reared in NE (barren) ($\chi^2 = 283.09$, df = 3, $P = 0.02$) tanks throughout the experimental period (Figure 6).

Physiological response of juveniles of *C. gariepinus*

The experiment consistently found the highest and least glucose values in PSE and SE tanks. At week two of the experiment, the EE treatments resulted in a slight increase in the glucose values obtained in the blood samples of *C. gariepinus* compared to the result obtained in week four across the treatments. By weeks six and eight, there was no significant difference in the glucose value recorded in all treatments. At the end of the experiment, the stress (glucose) level indicator showed that EE affected the level of glucose found in the blood of *C. gariepinus* during the study period ($\chi^2 = 36.55$, df = 3, $p = 0.01$) (Figure 7).

Discussion

This study examined the survival rate, growth indices, condition factors, behavioral traits and physiological response of juveniles of *C. gariepinus* exposed to different levels of environmental enrichment for 56-days. The findings of this research accepted this study's hypothesis. The hypothesis' prediction that the provision of physical forms of enrichment would improve the growth and survival rate of the juveniles of *Clarias gariepinus* and lower the frequency of aggressive acts and glucose response in their blood samples was satisfied.

In our study, the survival rate of juveniles of *C. gariepinus* was affected by the different forms of environmental

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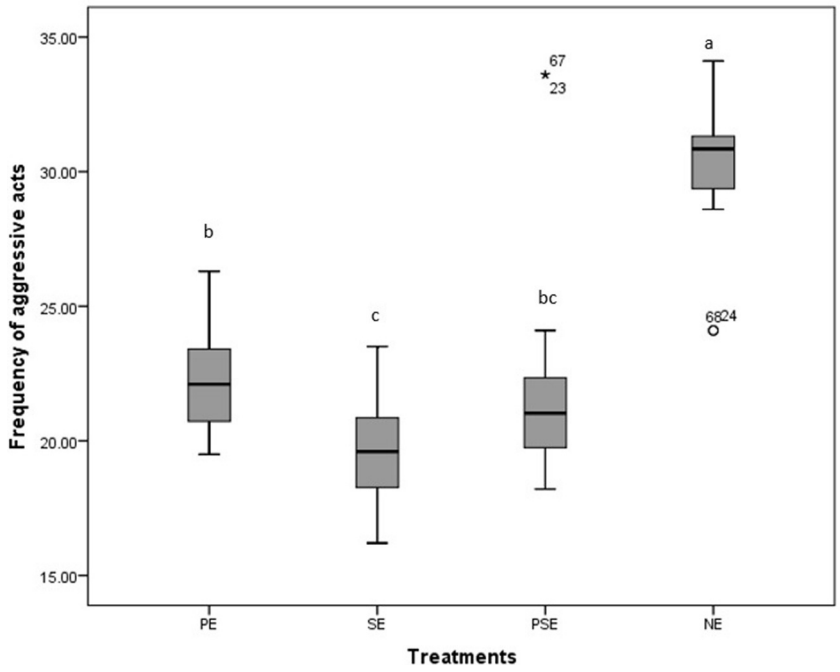

FIGURE 5
Boxplot of the effect of environmental enrichment during 600seconds on the frequency of aggressive acts displayed by juveniles of *Clarias gariepinus* under laboratory conditions. [abcd] Mean values with different superscripts were significantly ($p < 0.05$) different between treatments.

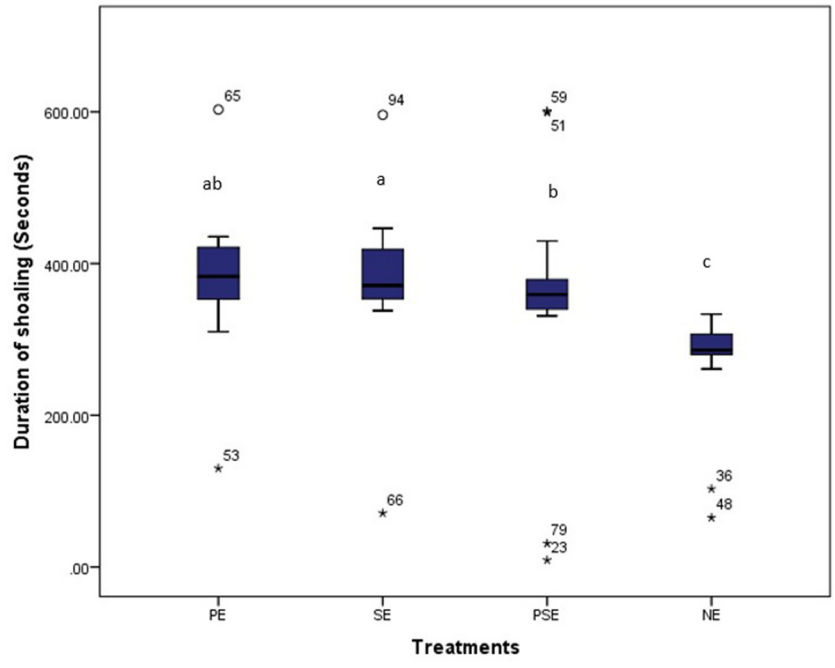

FIGURE 6
Average time (secs) spent by juveniles of *C. gariepinus* in displaying shoaling behaviour at plant enriched (PE), substrates enriched (SE), plant and substrates enriched (PSE) and non-enriched (NE) culture tanks. abcd Mean values with different superscripts were significantly ($p < 0.05$) different between treatments.

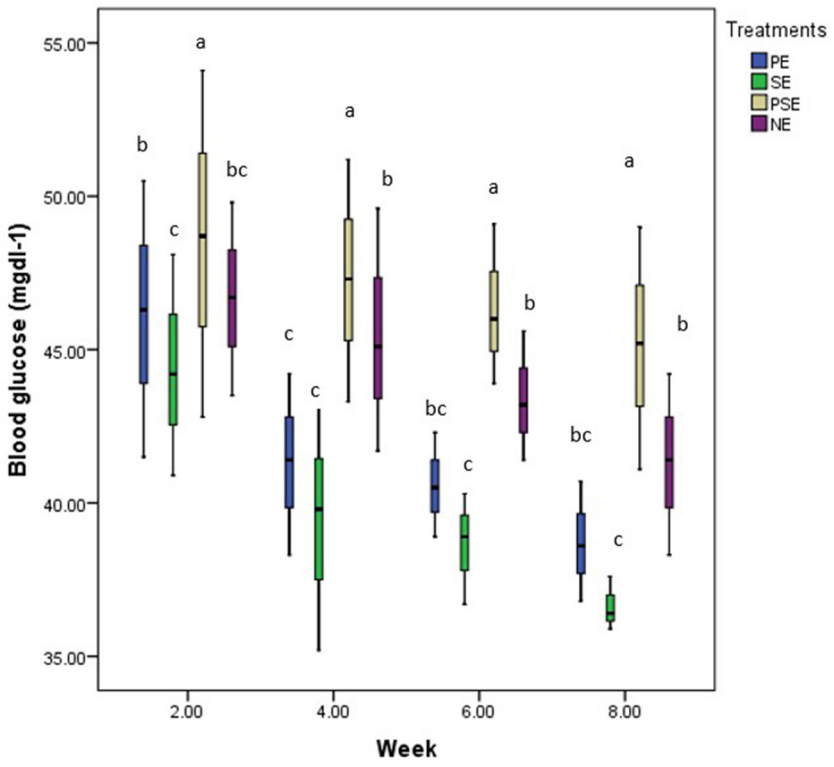

FIGURE 7
A boxplot showing the mean weekly glucose (mg/dl) values and bi-weekly trend of glucose in blood samples of *C. gariepinus* exposed to plant enriched (PE), substrates enriched (SE), plant and substrates enriched (PSE) and non-enriched (NE) culture tanks throughout the study period. [abcd] Mean values with different superscripts were significantly ($p < 0.05$) different between treatments.

enrichment, with the highest and least survival rates in SE and NE tanks, respectively. This observed variance in survival rates could be due to physical structures such as natural aquatic plants and substratum that aid water quality in their rearing enclosures. The provided physical enrichments further serve as hiding structures to prevent physical attacks, cannibalism and subsequent mortality, which was absent in the non-enriched tanks with the least survival rate. This finding agrees with the result of Lee et al. (9) and Boerrigter et al. (66), who reported a high survival rate in juveniles of *D. rerio* and African catfish exposed to the physical form of enrichments. However, the result of this study contradicts the findings of Arechavala-Lopez et al. (39), who reported that the survival rate of juveniles of *Sparus aurata* was not affected by the structural form of enrichment.

Fish growth represents a complex physiological process often affected by feed intake, feed metabolism, feed conversion rate and the health status of the fish species. It can also be described as an indicator of the biological functioning of the fish species in its culture environment (17, 24, 67). Growth could increase, remain static or decrease depending on the severity of the wellbeing or condition of the fish in its rearing enclosure. The mean weight gain of the juveniles of *C. gariepinus* in this study was similar at the beginning of the experiment. However, the higher mean weight gain found in SE, PSE and PE compared to the NE tanks suggests good feed metabolism, feed conversion rate and wellbeing in their enriched rearing enclosure (16, 68). This result corroborates the findings of Zhang et al. (12), Batzina and Karakatsouli (69), and Rosengren et al. (70), who reported a higher growth rate in juveniles of black rockfish, gilthead seabream and Atlantic Salmon exposed to physical enrichments compared to those reared in barren tanks. In addition, the higher mean weight gain in the enriched (PE, SE, PSE) tanks might be due to the fact that the provided enrichments suites the basic needs of the African catfish compared to the fish exposed to the barren tanks (NE) (9). However, the result of this study contradicts the findings of Boerrigter et al. (66), who noted a decrease in the feed response and growth of African catfish exposed to structural enrichments (PVC-tubes), which was attributed to the high stocking density used in the study.

The similar condition factor reported in *C. gariepinus* at the different enrichment levels at the beginning and end of the culture period showed that the condition factor of the fish species exposed to enriched and barren tanks was not compromised throughout the experiment. Moreover, the higher k-value in African catfish exposed to the SE form of

enrichment suggests that the provided form of enrichment met the requirements of the fish species in their rearing enclosures (18, 54).

This study found that *C. gariepinus* exposed to sediment enriched (SE) tanks took a shorter period to consume their diet. There were similarities in the duration used by African catfish exposed to PE and PSE to consume their feed ration. The observed similarities in the latency to feed displayed by fish in the SE and NE tanks compared to the PE and PSE tanks could be attributed to increased visibility which aids the zeal to feed and grow in the cultured fish species (40). This result is in line with the findings of Lee et al. (9) and Xu et al. (67) that reported an increase in the feed response and growth rate of zebrafish and rare minnows fish exposed to different forms of environmental enrichment. Moreover, the time of feeding during the day (morning vs. evening) had no significant effect on the feed response of *C. gariepinus* exposed to the different forms of environmental enrichment during the study (54). However, Zhang et al. (12) and Gregory and Wood (71) noted an inverse relationship between the presence of environmental enrichment and the feed response of juvenile Blackrock fish and rainbow trouts.

The environmental enrichments in this study affected the level of aggression displayed by the cultured juveniles of *C. gariepinus*. The least aggressive acts were recorded in SE tanks, probably because the fish shoals closer to the provided sediment than chasing or attacking each other within their rearing enclosure. This result corroborates the findings of Wilkes et al. (58), Boerrigter et al. (66), and Batzina and Karakatsouli (69), who reported decreased aggressive acts in *Danio rerio, C. gariepinus* and *Sparus aurata*. In addition, a relatively higher aggressive act was found in juveniles of *C. gariepinus* exposed to non-enriched tanks, which could be due to the barren nature of their rearing enclosure that aids visibility, frequency of encounter and the chances of establishing a territorial range within their tanks (44). This finding is similar to the result of Boerrigter et al. (66), who noted an increase in the aggression level of African catfish exposed to barren tanks in their study compared to the tanks enriched with PVC tubes. In addition, the frequency of aggressive acts was similar in PE and PSE tanks (40). Moreover, Arechavala-Lopez et al. (26) described environmental enrichment as a moderator of stress in fish by creating separate spaces to ease intraspecific aggression.

The observed higher shoaling rate displayed by juveniles of *C. gariepinus* close to the bottom of the water column or substratum found in fish exposed to SE tanks could be a defensive mechanism to discourage chases, unnecessary physical attacks, fights and injury to the fish. However, the duration of shoaling at the bottom of the tank enriched with PE, SE, and PSE did not vary throughout the culture period; this could be classified as an adaptive response for protection from predators (58). In addition, the similar duration of shoaling observed in these enriched tanks suggests an increased search for territory partners in their natural environment. This finding contradicts the result of Miller and Gerlai (59), who reported a decrease in the shoaling period of adult zebrafish exposed to physical forms of enrichment. However, the result of the present study agrees with the findings of Wilkes (57), who reported that zebrafish in enriched tanks shoaled more at the bottom of the tank compared to the same species reared in barren tanks.

Pankhurst (49) stated that poor conditions or impaired welfare in rearing enclosures are mostly accompanied by changes in the stress level of the fish. This stress level could be seen in blood parameters and other hormones, which might induce changes in a fish's survival, growth, behavior and physiology (51). The weekly trend of glucose levels found in the juveniles of *C. gariepinus* at the different forms of enrichment in this study further affirms the trend of aggressive acts displayed by the fish during the culture period. These findings implied that the SE form of enrichment is very beneficial for the welfare of *C. gariepinus* due to the reduced level of aggression and glucose recorded in the experiment. The consistent highest blood glucose over the culture period found in *C. gariepinus* exposed to PSE tanks could be a physiological process of adapting to and maintaining homeostasis in their internal environment.

Conclusion

Modifying the rearing enclosures of juveniles of *C. gariepinus* greatly improved the survival rate, mean weight gain, condition factor, behavioral and physiological response of *C. gariepinus* under laboratory conditions. Environmental enrichment of the rearing enclosure of African catfish with fine sand substratum gave the highest mean weight gain and least aggressive traits. In addition, the highest stress (glucose) level was found in non-enriched (barren) tanks. The result of this study has a significant implication for improving the production efficiency of this important aquaculture species for fish food security and sustainability. Thus, modification of rearing enclosures for juveniles of African catfish with physical structures could be applied in commercial settings to simulate natural behavior, improve the growth rate, and reduce the aggressiveness of the fish species.

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

The animal study was reviewed and approved by Animal Ethics and Welfare Committee of the Federal University of Agriculture, Abeokuta.

Author contributions

OO conceived the original idea, designed the methodology, wrote the manuscript, and supervised the execution of the project. MBO, CS, AB, and MO conducted the project. AA assisted with the blood collection and physiological analysis. SD did the statistical analyses. SD and IA proofread it. All authors contributed to the article and approved the submitted version.

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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.

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References

1. Finegold C. *The Importance of Fisheries and Aquaculture to Development. Fisheries, Sustainability and Development.* (2009). Stockholm: The Royal Swedish Academy of Agriculture and Forestry.

2. Adewunmi AA, Olaleye VF. Catfish culture in Nigeria: progress, prospects and problems. *Afr J Agric Res.* (2010) 6:1281–5. doi: 10.5897/AJAR09.361

3. Organisation FaA. *The State of World Fisheries and Aquaculture*. Rome: FAO (2012). p. 191.

4. FAO Organization FaA. *The State of the World Fisheries and Aquaculture. Meeting the Sustainable Development Goals.* (2018). p. 227.

5. Franks B, Ewell C. Animal welfare risks of global aquaculture. *Sci Adv.* (2021) 2:14. doi: 10.1126/sciadv.abg0677

6. Huntingford FA, Adams C, Braithwaite VA, Kadri S, Pottinger TG, Sandøe P, et al. Current issues in fish welfare. *J Fish Biol.* (2006) 68:332–72. doi: 10.1111/j.0022-1112.2006.001046.x

7. Amosu AO, Hammed AM, Togunde KA, Joseph OO, Adekoya A. Possible aquaculture development in Nigeria: evidence for commercial prospects. *J Agric Sci Technol*. (2017) B7:194–205. doi: 10.17265/2161-6264/2017.03.007

8. Ullah I, Zuberi A, Khan KU, Ahmad S, Thornqvist P, Winberg S. Effects of enrichment on the development of behaviour in anendangered fish mahseer (Tor putitora). *Appl Anim Behav Sci.* (2017) 186:93–100. doi: 10.1016/j.applanim.2016.10.016

9. Lee CJ, Paull GC, Tyler CR. Effects of environmental enrichment on survivorship, growth, sex ratio and behaviour in laboratory maintained zebrafish Danio rerio. *J Fish Biol.* (2018) 2018:1–10. doi: 10.1111/jfb.13865

10. Young RJ. *Environmental Enrichment for Captive Animals*. Oxford: BlackwellScience Ltd (2003). p. 228.

11. DePasquale C, Kemerer N, White N, Yost M, Wolfkill J, Sturgill J, et al. The influence of an enriched environment in enhancing recognition memory in zebrafish (danio rerio). *Front Vet Sci.* (2021) 8:749746. doi: 10.3389/fvets.2021.749746

12. Zhang ZH, Bai QQ, Xu XW, Guo HY, Zhang XM. Effects of environmental enrichment on the welfare of juvenile black rockfish Sebastes Schlegelii: growth, behavior and physiology. *Aquaculture.* (2020) 518:734782. doi: 10.1016/j.aquaculture.2019.734782

13. Adebayo I. Effect of photoperiod on eggs hatchability, growth and survivability of hybrid catfish (*Heterobranchus bidorsalis* x. *Clarias gariepinus). Larvae J Aquac Fisheries.* (2018) 2:1–7. doi: 10.24966/AAF-5523/100007

14. Oresegun A, Oguntade OR, Ayinla OA. A review of catfish culture in Nigeria. *Nigeria J Fish.* (2007) 2007:27–52.

15. Dauda AB, Natrah I, Karim M, Kamarudenn MS, Bichi AH. African catfish aquaculture in Malaysia and Nigeria: status, trends and prospects. *Fish Aquac J.* (2018) 9:1–7. doi: 10.4172/2150-3508.1000237

16. Fawole FJ, Adeoye AA, Tiamiyu LO, Ajala KI, Obadara SO, Ganiyu IO. Substituting fishmeal with Hermetia illucens in the diets of African catfish (Clarias gariepinus): Effects on growth, nutrient utilization, haemato-physiological response, and oxidative stress biomarker. *Aquaculture.* (2020) 518:734849. doi: 10.1016/j.aquaculture.2019.734849

17. Adeoye AA, Obasa SO, Fawole FJ, Wan AHL, Davies SJ. Dietary supplementation of autolyzed yeast enhances growth, liver functionality and intestinal morphology in African catfish. *Aquac Nutr.* (2020) 26:772–80. doi: 10.1111/anu.13036

18. Martins CIM, Trenovski M, Schrama JW, Verreth JAJ. Comparison of feed intake behaviour and stress response in isolated and non-isolated African catfish. *J Fish Biol.* (2006) 69:629–36. doi: 10.1111/j.1095-8649.2006.01121.x

19. Almazán-Rueda P, Schrama JW, Verreth JAJ. Behavioural responses under different feeding methods and light regimes of the African catfish (*Clarias gariepinus*) juveniles. *Aquaculture.* (2004) 231:347–59. doi: 10.1016/j.aquaculture.2003.11.016

20. Assan D, Anane K, Abarike ED, Alhassan EH, Ampofo-Yeboah A. Evaluation of induced breeding of catfish (Clarias gariepinus), using different doses of normal saline diluted ovaprim. *J Appl Aquac.* (2020) 2020:1–13. doi: 10.1080/10454438.2020.1866142

21. Martins CIM, Schrama JW, Verreth JAJ. The effect of group composition on the welfare of African catfish (*Clarias gariepinus*). *Appl Anim Behav. Sci.* (2006) 97:323–34. doi: 10.1016/j.applanim.2005.07.003

22. Nieuwegiessen PG, van de B, Verreth JAJ, Schrama JW. Assessing the effects of a chronic stressor, stocking density, on welfare indicators of juvenile African catfish, *Clarias gariepinus* Burchell. *Appl Anim Behav. Sci.* (2008) 115:233–43. doi: 10.1016/j.applanim.2008.05.008

23. Nieuwegiessen PG, van de OJ, Khong S, Verreth JAJ, Schrama JW. Effects of age and stocking density on the welfare of African catfish, *Clarias gariepinus* Burchell. *Aquaculture*. (2009) 288:69–75. doi: 10.1016/j.aquaculture.2008.11.009

24. Hossain MAR, Beveridge MCM. The effects of density, light and shelter on the growth and survival of African catfish (*Clarias gariepinus* Burchell, 1822) fingerlings. *Aquaculture*. (1998) 160:251–8. doi: 10.1016/S0044-8486(97)00250-0

25. Schram E, Schelvis R, Heul J, Luten J. Enrichment of the African catfish *Clarias gariepinus* (Burchell) with functional selenium originating from garlic: effect of enrichment period and depuration on total selenium level and sensory properties. *Aquac Res.* (2009) 41:793–803. doi: 10.1111/j.1365-2109.2009.02356.x

26. Arechavala-Lopez P, Cabrera-Alvarez MJ, Maia CM, Saraiva JL. Environmental enrichment in fish aquaculture: a review of fundamental and practical aspects. *Rev Aquacult.* (2021) 14:704–28. doi: 10.1111/raq.12620

27. Duncan IJH. Science-based assessment of animal welfare: farm animals. *Rev Sci Tech OIE.* (2005) 24:483–92. doi: 10.20506/rst.24.2.1587

28. Duncan IJH. The changing concept of animal sentience. *Appl Anim Behav Sci.* (2006) 100:11–9. doi: 10.1016/j.applanim.2006.04.011

29. Giménez-Candela T, Saraiva JL, Bauer H. The legal protection of farmed fish in Europe: analyzing the range of EU legislation and the impact of international animal welfare standards for the fishes in European aquaculture. *dA Derecho Anim Forum Anim Law Stud.* (2020) 11:65–118. doi: 10.5565/rev/da.460

30. Saraiva JL, Arechavala-Lopez P. Welfare of fish—no longer the elephant in the room. *Fishes* (2019) 4:39. doi: 10.3390/fishes4030039

31. Sneddon LU, Lopez-Luna J, Wolfenden DCC, Leach MC, Valentim AM, Steenbergen PJ, et al. Fish sentience denial: muddying the waters. *Anim Sentience.* (2018) 3:21–29. doi: 10.51291/2377-7478.1317

32. Brown C. Fish intelligence, sentience and ethics. *Anim Cogn.* (2014) 18:1–17. doi: 10.1007/s10071-014-0761-0

33. Mason GJ, Lavery JM. What is it like to be a bass? red herrings, fish pain and the study of animal sentience. *Front Vet Sci.* (2022) 9:788289. doi: 10.3389/fvets.2022.788289

34. Conte FS. Stress and the welfare of cultured fish. *Appl Anim Behav Sci.* (2004) 86:205–23. doi: 10.1016/j.applanim.2004.02.003

35. Ashley PJ. Fish welfare: current issues in aquaculture. *Appl Anim Behav Sci.* (2007) 104:199–235. doi: 10.1016/j.applanim.2006.09.001

36. Gerber. B, Stamer A, Stadtlander T. Environmental enrichment and its effect on fish welfare: review. *Excellence Sustain.* (2015) p. 74.

37. Kristiansen TS, Fernö A, Pavlidis MA. *The Welfare of Fish*. Springer: Switzerland (2020). p. 515.

38. Brydges NM, Braithwaite VA. Does environmental enrichment affect the behaviour of fish commonly used in laboratory work? *Appl Anim Behav Sci.* (2009) 118:137–43. doi: 10.1016/j.applanim.2009.02.017

39. Arechavala-Lopez P, Diaz-Gil C, Saraiva JL, Moranta D, Castanheira MF, Nuñez-Velázqueza S, et al. Effects of structural environmental enrichment on welfare of juvenile seabream (*Sparus aurata*). *Aquacult Rep.* (2019) 15:100224. doi: 10.1016/j.aqrep.2019.100224

40. Naslund J, Johnson JI. Environmental enrichment for fish in captive environments: effects of physical structures and substrates. *Fish Fisheries.* (2016) 2016:1–30. doi: 10.1111/faf.12088

41. Brunet V, Kleiber A, Patinote A, Sudan PL. Positive welfare effects of physical enrichments from the nature-, functions- and feeling- based approaches in farmed rainbow trout (Oncorhynchus mykiss). *Aquaculture.* (2022) 550:737825. doi: 10.1016/j.aquaculture.2021.737825

42. Villamagna AM, Murphy BR. Ecological and socio-economic impacts of invasive water hyacinth (*Eichhornia crassipes*): a review. *Freshw Biol.* (2010) 55:282–98. doi: 10.1111/j.1365-2427.2009.02294.x

43. Echiegu E, Ezeugwu L, Ugwu S. Effects of water hyacinth (*Eichhornia crassipes*) on the physicochemical properties of fishpond water and growth of African catfish. *Afr J Agric Res.* (2018) 13:54–66. doi: 10.5897/AJAR2017.12794

44. Zhang Z, Fu Y, Zhang Z, Zhang X, Chen S. A comparative study on two territorial fishes: the influence of physical enrichment on aggressive behavior. *Animals.* (2021) 11:1868. doi: 10.3390/ani11071868

45. Torrezani CS, Pinho-Neto CF, Miyai CA, Sanches FHC, Barreto RE. 183-190. Structural enrichment reduces aggression in Tilapia rendalli marine and freshwater. *Behav Physiol.* (2013) 46:183–90. doi: 10.1080/10236244.2013.805053

46. Jentoft S, Aastveit AH, Torjesen PA. Effects of stress on growth, cortisol and glucose levels in non-domesticated Eurasian perch (Perca fluviatilis) and domesticated rainbow trout (*Oncorhynchus mykiss*). *Comp Biochem Physiol A Mol Integr Physiol.* (2005) 141:353–8. doi: 10.1016/j.cbpb.2005.06.006

47. Brown J, Walker S, Steinman K. *Endocrine Manual for the Reproductive Assessment of Domestic and Non-Domestic Species*. Endocrine Research Laboratory, Department of Reproductive Sciences, Conservation and Research Center, National Zoological Park, Smithsonian Institution, Handbook. An Open Access Publisher, Virginia United State (2004). p. 64.

48. Broom D. *Domestic Animal Behaviour and Welfare*. Published by Wallingford, CABI, United Kingdom (2015). p. 438.

49. Pankhurst NW. The endocrinology of stress in fish: an environmental perspective. *Gen Comp Endocrinol.* (2011) 170:265–75. doi: 10.1016/j.ygcen.2010.07.017

50. David M, Shivakumar R, Mushigeri SB, Kuri RC. Blood glucose and glycogen levels as indicators of stress in the freshwater fish, *Labeo rohita* under fenvalerate intoxication. *J Ecotoxicol Environ Monit.* (2005) 15:1–5.

51. Endo H, Wu H. Biosensors for the assessment of fish health: a review. *Fish Sci.* (2019) 85:641–54. doi: 10.1007/s12562-019-01318-y

52. Malini DM, Madihah AF, Arista S. Increased blood glucose level on pelagic fish as response to environmental disturbances. *IOP Conf Ser Earth Environ Sci.* (2018) 166:201–11. doi: 10.1088/1755-1315/166/1/012011

53. Dawkins MS. Using behaviour to assess animal welfare. *Anim Welfare.* (2004) 13:3–7.

54. Ojelade O, Iyasere O, Durosaro S, Abdulraheem I, Akinde A. Social isolation impairs feed intake, growth and behavioural pattern of catfish under culture conditions. *Animal.* (2022) 16:100521. doi: 10.1016/j.animal.2022.100521

55. Martins CI, Galhardo L, Noble C, Damsgård B, Spedicato MT, Zupa W, et al. Behavioural indicators of welfare in farmed fish. *Fish Physiol Biochem J.* (2012) 38:17–41. doi: 10.1007/s10695-011-9518-8

56. Salvanes A, Moberg O, Braithwaite V. Effects of enriched environment on group behaviour in fish. *Anim Behaviour*. (2007).74:805–11. doi: 10.1016/j.anbehav.2007.02.007

57. Wilkes L. *Potential Environmnetal Enrichment of Zebrafish used in Regulatory Toxicology. A PhD Thesis Submitted to University of Exeter.* (2011). p. 295.

58. Wilkes L, Owen SF, Readman GD, Sloman KA, Wilson RW. Does structural enrichment for toxicology studies improve zebrafish welfare? *Appl Anim Behav Sci.* (2012) 139:143–50. doi: 10.1016/j.applanim.2012.03.011

59. Miller NY, Gerlai R. Oscillations in shoal cohesion in zebrafish (Danio rerio). *Behav Brain Res.* (2008) 193:148–51. doi: 10.1016/j.bbr.2008.05.004

60. Rosburg AJ, Fletcher BL, Barnes ME, Treft CE, Bursell BR. Vertically suspended environmental enrichement structures improve the growth of juvenile landlocked fall chinook Salmon. *Int J Innov Stud Aquatic Biol Fisheries.* (2019) 5:17–24. doi: 10.20431/2454-7670.0501004

61. White SC, Krebs E, Huysman N, Voorhees JM. Use of suspended plastic conduit arrays during brown trout and rainbow trout rearing in circular tanks. *N Am J Aquac*. (2019) 81:101–6. doi: 10.1002/naaq.10076

62. Dauda AB, Romano N, Ebrahimi M, Karim M, Natrah I, Kamarudin MS, et al. Different carbon sources affects biofloc volume, water quality and the survival and physiology of African catfish *Clarias gariepinus* fingerlings reared in an intensive biofloc technology system. *Fisheries Sci.* (2017) 83:1037–48. doi: 10.1007/s12562-017-1144-7

63. Adeoye AA, Yomla R, Jaramillo-Torres A, Rodiles A, Merrifield DL, Davies SJ. Combined effects of exogenous enzymes and probiotic on Nile tilapia (*Oreochromis niloticus*) growth, intestinal morphology and microbiome. *Aquaculture.* (2016) 463:61–70. doi: 10.1016/j.aquaculture.2016.05.028

64. LeCren. The length-weight relationship and seasonal cycle in gonad weight and condition in Perch, Perca fluviatilis. *J Anim Ecol.* (1951) 20:201–19. doi: 10.2307/1540

65. Di Marco P, Priori A, Finoia MG, Massari A, Marino G. Physiological responses of European sea bass Dicentrarchus labrax to different stocking densities and acute stress challenge. *Aquaculture.* (2007) 275:319–28. doi: 10.1016/j.aquaculture.2007.12.012

66. Boerrigter JG, Bos R, Vis H, Spanings T, Flik G. Effects of density, PVC-tubes and feeding time on growth, stress and aggression in African catfish (*Clarias gariepinus*). *Aquac Res.* (2016) 47:2553–68. doi: 10.1111/are.12703

67. Xu C, Hou M, Su L, Qiu N, Yu F, Zou X, et al. The effect of environmental enrichment on laboratory rare minnows (*Gobiocypris rarus*): growth, physiology, and behavior. *Animals.* (2022) 12:514. doi: 10.3390/ani12040514

68. Appelbaum S, Kamler E. Survival, growth, metabolism, and behaviour of *Clarias gariepinus* (Burchell 1822) early stages under different light conditions. *Aquacult Eng.* (2000) 22:269–87. doi: 10.1016/S0144-8609(00)00054-6

69. Batzina A, Karakatsouli N. The presence of substrate as a means of environmental enrichment in intensively reared gilthead seabream *Sparus aurata*: growth and behavioral effects. *Aquaculture*. (2012) 370–1:54–60. doi: 10.1016/j.aquaculture.2012.10.005

70. Rosengren M, Kvingedal E, Näslund J, Johnsson JI, Sundell K. Born to be wild: effects of rearing density and environmental enrichment on stress, welfare, and smolt migration in hatchery-reared Atlantic salmon. *Can J Fish Aquat Sci*. (2016) 74:396–405. doi: 10.1139/cjfas-2015-0515

71. Gregory TR, Wood CM. The effects of chronic plasma cortisol elevation on the feeding behaviour, growth, competitive ability, and swimming performance of juvenile rainbow trout. *Physiol Biochem Zool*. (1999) 72:286–95. doi: 10.1086/316673

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Mouse breeding facilities in Argentina: Current state, challenges, and strengths in relation to animal welfare

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The science and technology of laboratory animals has come a long way worldwide, but for reasons related to the development of the countries, this journey started later in some Latin American countries, as is the case of Argentina. Without a specific legal framework to conduct animal experimentation, local strengths to promote animal welfare are based on professionals specifically trained in the care of laboratory animals as well as an extended network of ethics committees that ensures compliance with the ethical principles applied to animal experimentation. Nevertheless, there are no updated reports showing welfare indicators in rodent facilities. Therefore, we conducted a survey on mice breeding facilities enrolled in a national record elaborated by the National Ministry of Science. Questions related to four of the Five Domains Model of Mellor, concerning (1) nutrition, (2) physical environment, (3) health, and (4) behavioral interactions with the environment, other animals, and humans, were included as well as information concerning general aspects of the establishments. Data obtained from 25 mice breeder facilities localized all over the country were summarized, providing for the first time a clear picture of the national situation about the welfare of laboratory mice in these establishments. This data will be essential to design future policy as well as for deciding priorities aiming to improve the welfare of mice bred in Argentinian facilities.

KEYWORDS

mice, animal facility, animal welfare, Latin America, Argentina, breeding

Introduction

In most countries, particularly in developed countries from the global north, minimum requirements for laboratory animals are strictly regulated by specific legislation (1). For example, the UK passed the Animals (Scientific Procedures) Act in 1986, the member states of the Council of Europe must follow Directive 2010/63/EU on the protection of animals used for scientific purposes, the US follows the Guide for the Care and Use of Laboratory Animals (2), while in Canada legislation regarding

animal research falls under provincial jurisdiction (3). Particularly, Latin America, Brazil, Mexico, and Uruguay have recently implemented specific legislation regarding the use of laboratory animals. Nevertheless, the current picture in this part of the globe is heterogeneous and frequently linked to the economic and political status of each particular country (4).

In Argentina, the situation of Laboratory Animal Science is not disconnected from the state of affairs in the country. In this sense, we have recently identified the strengths and difficulties in sight of the development of this scientific discipline (5). Regarding the legislation, Argentina has a law that protects animals against cruelty acts (Law 14346, proclaimed in 1954) but does not have a specific law that regulates scientific procedures in laboratory animals. Moreover, the modern view considers that to promote an optimal welfare state, minimum requirements must be surpassed by including appropriate refinements to the housing or husbandry protocols (1). Despite this difficult scenario, one can be optimistic because the standards for animals used in experiments conducted in Argentina are set by an extensive network of institutional ethics committees that oversee the experimental protocols in accordance with international recommendations (5). Nevertheless, the requirements for breeding laboratory animals are less regulated, which is problematic since in countries with specific regulations and statistics, at least three additional animals are needed for every two animals employed in experimentation (6).

One of the main drawbacks of not having a specific law that oversees animal experimentation is that there are no local statistics, so the extent to which modern refinements have been incorporated into the different animal facilities is currently unknown. Hence, the main objective of the present work is to characterize the current situation of the breeding facilities in Argentina, in order to identify its strengths as well as areas in which animal welfare might be compromised.

The first step is to define how to evaluate the welfare of the laboratory mice in the breeding facilities. In general, Animal Welfare Science aims to assess, through objective indicators, the subjective perception that an animal has of its own quality of life (7). This is clearly challenging since it involves the selection of appropriate markers across scientific disciplines (8), which can even include indirect markers such as those related to the environment. To address this problem, a framework based on five domains was first proposed by Mellor in 1994 (9), which was frequently revised and extended afterwards to ensure that the recommendations were up to date with the latest literature (10). Succinctly, the five domains model currently comprises: (1) Nutrition, including the quality and availability of the food and water supply; (2) Physical Environment, which consists of the enclosure's characteristics per se as well as the quality of the resources such as the air, light, and noise; (3) Health, considering disease due to pathological agents, poisoning, husbandry/experimental procedures that may cause pain or discomfort, among other things; (4) Positive and negative behavioral interactions with the environment, with other animals, and with humans. (5) Mental state, i.e., the affective processes derived from the previous four domains (e.g., feeling hungry due to an inappropriate supply of food). The first four domains can be assessed by direct observation of the animals or their environments, while the fifth domain would require specific assays to measure them indirectly [e.g. judgment bias task to assess positive or negative affective states triggered by enriched or standard housing, respectively (11)]. Therefore, we developed a questionnaire based on these first four domains to characterize, as described above, the breeding facilities in Argentina. Our samples were the institutions enrolled in the 'Sistema Nacional de Bioterios' (SNB), a national record of animal facilities set by the National Ministry of Science, to which establishments adhere voluntarily. Overall, we expect that this information allows us to depict the actual animal welfare situation, as well as to propose future strategies to improve animal welfare, according to the reality of the region.

Materials and methods

Sample

We targeted institutions breeding mice that are voluntarily enrolled in the SNB, a national record of animal facilities set by the National Ministry of Science. Establishments adhere voluntarily to this registry, which allows them to access specific funding schemes. We identified 46 candidate institutions, and they were contacted *via* the email address that was noted in the national registry.

Survey

The survey was conducted in Google Forms, consisting of 75 questions divided into five sections, and institutions participated on a voluntary basis. The full translated version can be seen in the Supplemental materials but briefly, the first section encompassed general questions about the size of the facility, species that they breed, type of records, genetic origin, and quality of the mouse colony. The remaining four sections were based on Mellor's Five Domains Model (10), considering the items that could be assessed by direct observation of the animals or their environment. First, the Nutrition domain was assessed, determining whether food and water were freely available, if these were treated to reduce the risk of microbiological contamination, if conditions such as over- or underweight are frequently observed [measured with the body condition scoring scale, a system that was adapted for the laboratory mouse (12)], and if unforeseen events, such as empty water bottles, have been recently detected. The following section considered the

Environment, inquiring about the type of housing that is used, the control over the environmental conditions (such as the room temperature), the basic resources that are provided to the animals, the adverse effects of these resources, and the capacity of the institution to resolve unforeseen events (e.g. due to the presence of contingency plans). The third segment was about the health status of the colony. Here, we asked about microbiological monitoring, the presentation of certain health conditions, as well as preventive treatments. Finally, the behavioral interaction with the environment (in particular about the administration of environmental enrichment), with other animals, and with the personnel were assessed (the type of training/continuous education of the workers, the methods for handling, and the consequences of these interactions, i.e., if biting happened recently). The collected data was summarized and anonymously reported in the Results, according to the different five sections.

Results

Ten out of the 46 institutions enrolled in the SNB did not answer the survey. Of the remaining 36 institutions, 11 were not included in the results since they declared that they do not breed laboratory mice. Therefore, for the data analysis, 25 complete forms from institutions that breed mice were processed. Results are presented according to the five sections of the survey.

General description

The laboratory mouse is the sole species bred in 11 institutions, whereas half of the facilities that filled the form breed rats besides mice. Additionally, six facilities breed less common species (e.g. rabbits). The most popular strains among the 25 institutions (Figure 1A) are related to the C57BL/6 and the BALB/c families. Indeed, C57 and BALB/c mice are bred in 88% and 80% of the facilities, respectively. However, in most of them, proper nomenclature or even the origin of mice, are not properly defined/known. Concerning available outbred stocks, CF1, Swiss, and NOD are present in four, two, and two facilities, respectively. Transgenic lines are also bred in six institutions. Most institutions have one or two rooms specifically devoted to mice breeding (Figure 1B), whereas the number of technicians devoted to the work with animals is quite variable among the institutions (Figure 1C). In 64% of the facilities, technicians are specifically devoted to animal-related labor, whereas in the rest, technicians rotate between different tasks. Except for one facility, single species are maintained in the same room, but in most of them (80%), different mice strains are kept in the same room. Concerning quarantine rooms, 72% of facilities have one. Regarding the acquisition of mice, two-thirds of the institutions acquired the different strains less than five years ago (Figure 1D), but this information is misleading since most facilities bought their breeders from local facilities whose colonies were acquired longer ago. Therefore, if the original provenance of mice is taken into account, these proportions are reversed (Figure 1E), with almost two-thirds of the facilities acquiring their colonies more than 20 years ago. In this sense, 80% of facilities have directly or indirectly acquired their colonies from Jackson or Charles River Laboratories. One-fifth of the institutions produced less than 1,000 mice per year (Figure 1F) and except for one facility that is devoted to quality control, more than 50% of mice produced are employed in research projects. Breeding records are kept in all the facilities except one, consisting of written records in 28% of the cases, and mixed (written and digital) in the remaining institutions. Genetic quality is controlled only in four facilities, three of which are the main mice suppliers to the rest of the institutions mentioned above. Two facilities have sent samples abroad to control the genetic quality, whereas the other two have analyzed their mice colonies in a local laboratory. Although only two establishments mention not following a specific breeding system, the breeding methods for inbred strains/outbred stocks were not properly defined in most of them. It is also interesting to note that two institutions specified that it was difficult for them to maintain the outbred status of their colonies.

Nutritional status

All the establishments provide *ad libitum* water, with two-thirds of them applying a treatment to reduce the microbiological count. Results concerning the frequency of findings of flooded cages due to malfunction of water systems or of empty bottles are presented in Figure 2A, B, respectively. Regarding feeding, all establishments provide food *ad libitum*, and only half of them apply a treatment to reduce the microbiological count. In 39% of facilities, food supplementation is included as an enrichment strategy to improve breeders' performance, or to compensate for deficiencies in the rodent chow. During the last month, four facilities reported having witnessed body condition below the ideal scoring of 3 and they were able to identify the reason. Also, five facilities declared having found mice with body conditions above this ideal scoring. In Argentina there are two local producers of food for mice and rats: Asociación de Cooperativas Argentinas (ACA) and Grupo Pilar S.A. (GEPSA), and therefore, all the facilities use one of these brands or even a mix of both. At least 10 establishments reported the regular presence of dust in the food, variable characteristics, and even the presence of insects, independently of the brand.

Environment

Half of the institutions maintain mice in opaque cages that impair continuous observation of animals inside the enclosure.

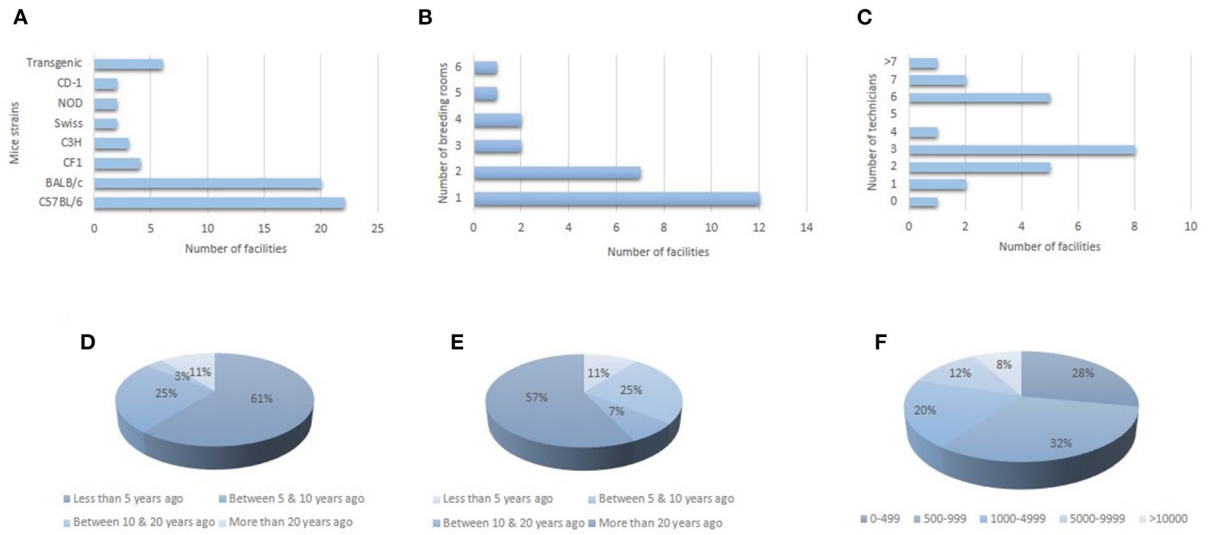

FIGURE 1
Characteristics of the breeding colonies and facilities, including the number of people devoted to the care of the animals. **(A)** Frequency of inbred strains and outbred stocks in the different institutions. **(B)** Number of rooms that facilities devote to breeding mice. **(C)** Number of technicians available to supervise mice. **(D)** Date of first acquisition of breeders. **(E)** Real age of mice strains, taking into account when breeders were first imported to the country. **(F)** Number of mice produced per year in each institution.

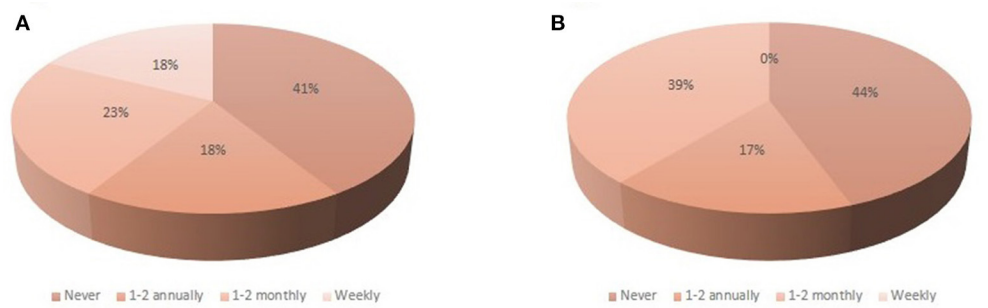

FIGURE 2
Overview of the situations associated with the supply of water. **(A)** Frequency of flooded cages due to malfunction of water bottles. **(B)** Frequency in which empty bottles are found during cleaning routines.

According to the different answers, these cages have been and continue to be replaced by transparent ones, but since it implies an important expense, it will still take time to discard the opaque cages. Economic reasons are also at the base of the fact that around 75% of facilities still have open-top cages (Figure 3A). From those establishments with individually ventilated cages (IVC), all except one manipulate animals inside a change station. Lesions in the animals due to the cage design have been noticed in four establishments. Concerning environmental parameters, temperature is maintained constant in all the facilities by means of different systems (Figure 3B), and positive pressure between the rooms and the corridors (a strategy to avoid/reduce microbiological contamination) is maintained in 56% of the establishments. In addition, with the exception of two facilities, all institutions have air extractors installed in the animal rooms, resulting in very few reports of personnel suffering (mucous membranes) irritation caused by ammonia accumulation (Figure 3C). All the establishments work under white light in the rooms. When cleaning the cages, bedding is barely dumped/wet in most cases (Figure 3D). With respect to the bedding, 80% of the establishments use wooden shavings, 16% employ corncob, and the rest, a mixture of both materials. Despite the fact that 76% of the facilities treat the bed material to reduce the microbiological count, only 44% treat the bedding to reduce the dust. Noises in the rooms can be heard from outside in 28% of the facilities. Finally, whereas 68% of the institutions

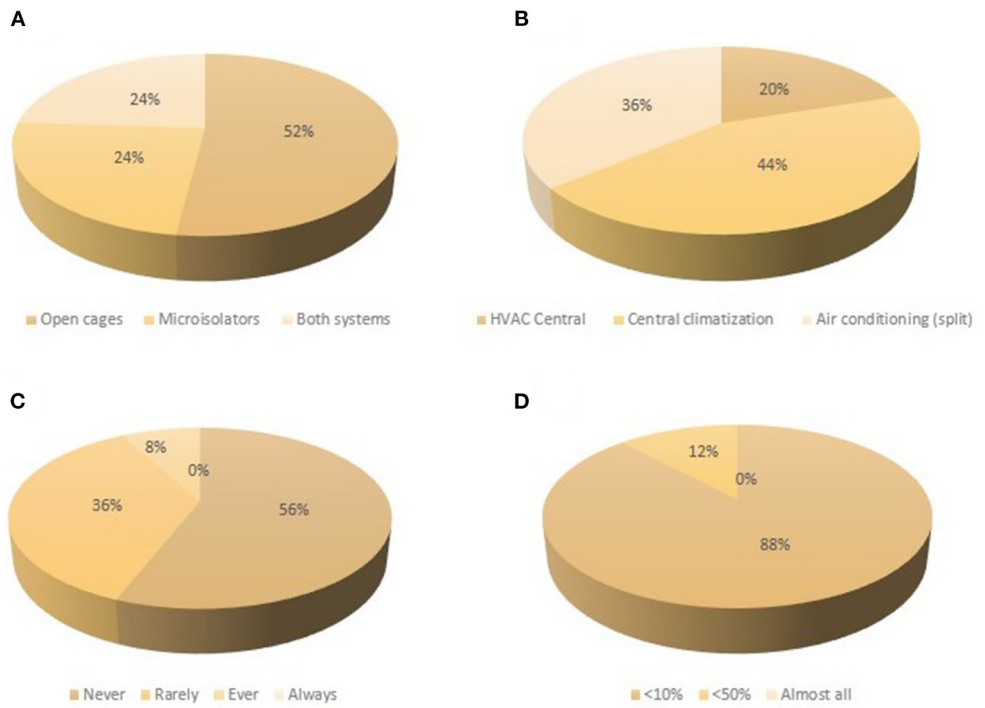

FIGURE 3
Summary of the environmental conditions that the animals are exposed to. **(A)** Type of cages held in animal facilities. **(B)** Heating, ventilation, and air conditioning (HVAC) systems. **(C)** Frequency of mucous membranes irritation experienced by the personnel when entering the animals' rooms. **(D)** Presence of fully dumped cages when changed.

count with an emergency power generator, only 20% of them count with an emergency contingency plan in case evacuation is needed.

Health status

Although not mandatory, 92% of institutions have an Attending Veterinarian. According to the frequency of monitoring, institutions were grouped as shown in Figure 4A, with almost one-third of facilities not controlling the microbiological status of their colonies. Among the institutions that perform regular microbiological monitoring, six of them send their samples to the Laboratory of Experimental Animals (LAE), Faculty of Veterinary Sciences, National University of La Plata, whereas two of them send their samples abroad (to Charles River Laboratories). All of them assess bacteria, virus, fungi, and parasites. The rest of the establishments (11) analyze their colonies in local laboratories in which not all of the mentioned agents are studied, yielding incomplete microbiological status profiles. Therefore, although six facilities declare themselves as Specific-Pathogen-Free (SPF) and 12 as conventional (Figure 4B), the agents controlled according to their own reports are not adequate to declare that status (13). In 44% of facilities, treatments against parasites are applied, either preventively or after positive results. Concerning adverse situations in mice' cages, the conditions reported more frequently are barbering and cannibalism, followed by perinatal mortality (Figure 4C).

Behavioral interactions

Interactions with the environment

Besides bedding, water, and food, enrichment elements are commonly added to the cages as shown in Figure 5A. While 20% of facilities change enrichments depending on the type of animal (usually, increasing enrichment in reproduction cages), 20% of facilities also vary enrichment elements along the year. No institution reported adverse effects due to the incorporation of environmental enrichment. Only three establishments declared having found an animal outside of its cage.

Interactions with other animals

Except for three facilities, animal groups were maintained after weaning. Five facilities reported hearing audible vocalizations that may be indicative of fights between animals.

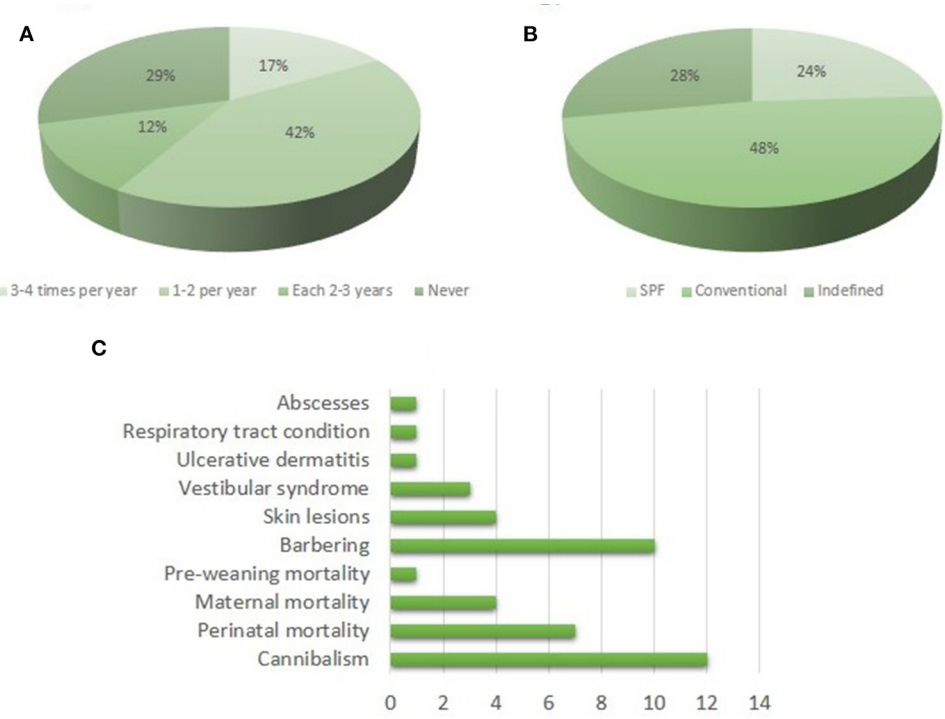

FIGURE 4
Overview of the health status of the breeding colonies. **(A)** Frequency of microbiological controls. **(B)** Type of microbiological status. **(C)** Prevalence of the common pathological findings.

Whereas 28% of establishments keep certain mice categories single-housed, during the last month, 32% of them had to separate already established groups due to fighting.

Interactions with the people

Sixty percent of the facilities have personnel attending during weekends. With respect to the type of continuous education, distribution is shown in Figure 5B. Ten facilities employ non-aversive methods to manipulate mice, and it is always combined with tail handling. Only in five institutions, the personnel is not stable for the same group of animals. During the last month, no facility has reported any mice biting the personnel.

Discussion

In the present work, we describe the current state of mouse breeding facilities in Argentina. There are a number of caveats associated with the idiosyncrasy of the country and the restrictions (due to economic and bureaucratic reasons, among other things) that researchers, technicians, and facility managers have to face on a day-to-day basis. Here, we have described the current infrastructure and husbandry of breeding facilities, which are at the core of animal research.

It is important to remark that no international certified breeders (such as Charles River, Jackson, Harlan, or Taconic Laboratories) have facilities neither in our country nor in any other Latin American country. Alternatively, there are three facilities in Argentina that provide mice with certified genetic and microbiologic quality. This fact is key to understanding the dynamics of our facilities, as many of the smaller suppliers have first obtained their breeders from these bigger institutions, a situation already described in a previous report (14). This could also be influencing the low availability of different strains in the country, with most facilities breeding mice from the C57 and BALB/c families. This is in line with the fact that they are the most commonly used strains, but considering that international suppliers are not readily available in the country, it certainly restricts the possibilities of researchers. Indeed, it would be important to envision a plan that brings less profitable strains for specific research protocols that have a national interest (e.g. to study endemic diseases).

From this survey, it was possible to identify that the average animal facility in Argentina uses open-top cages with wood shavings and some sort of nest material. Although this might not be the trend in Laboratory Animal Science (which consists of IVC cages with corncob bedding), there are reports

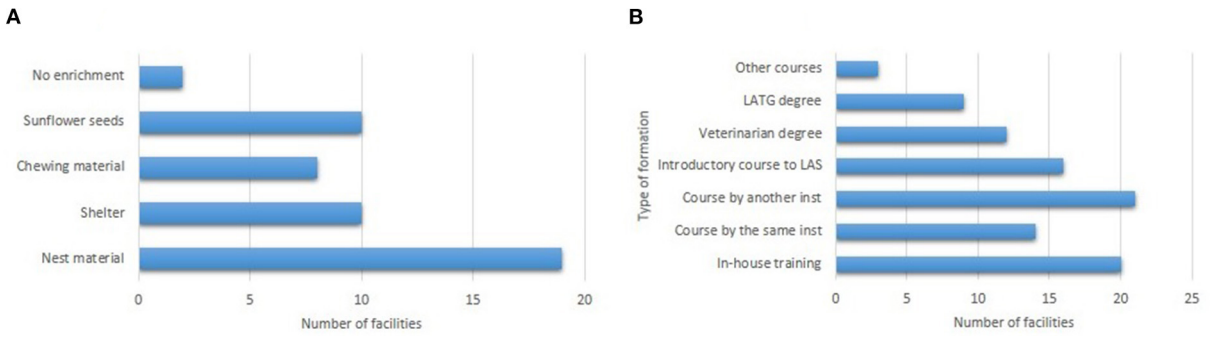

FIGURE 5
Outline of the factors which mice interact with. **(A)** Type of enrichments that facilities include to the cages of the breeding colonies. **(B)** Type of training that the personnel receives.

suggesting that these local conditions could be better for animal welfare. For example, the literature suggests that IVC cages can induce chronic cold stress (15), and males housed in these cages (especially when corncob bedding is used) tend to fight more (16). Corncob bedding is usually preferred among animal technologists because it reduces the spread of allergens (17) and the ammonia levels inside the cages (18). Nevertheless, the presentation of high levels of ammonia in the animals' rooms—as perceived by the personnel- is not preponderant among the institutions surveyed, and wood shavings—in comparison to corncob bedding- are always preferred by the mice (19). Moreover, as the presence of dumped bedding in the cages when cleaning is rare, this type of bedding seems to have adequate absorbance. Still, institutions should increase their efforts to improve the quality of the bedding. This can be done relatively easily by sieving the wood shavings (this is currently done in less than half of the facilities) and sterilizing them with any method available.

In accordance with standard practice generally adopted across mouse facilities worldwide, food and water are administered *ad libitum*. Together with the fact that all facilities report the use of standard cages which largely limits the possibility of exercising, the finding of overweight in some facilities could be expected. Paradoxically, underweight mice (with body condition scoring below three) were also detected in several facilities. This is a complicated issue to address since specific diets are not easily available in the country (e.g. low fat), so diet imbalances in some of the animal categories could be expected. This problem is worsened by the fact that some serious quality issues, such as the presence of insects, were noted by some institutions. The presence of empty water bottles is a rare event, but the occurrence of flooding in the cages is relatively common, again potentially due to the quality of the water bottles that are available in the animal facilities.

All institutions use white lights, but given that opaque cages are still widely implemented (half of the institutions) and the fact that some sort of protection from the direct light is usually provided (either by providing nest material or shelter), we do not expect that this condition would be particularly aversive for the mice. Cleaning routines under white light might alter their circadian rhythm (20), but in general, this happens only once a week in breeding facilities. Although using an inverted light cycle can reduce anxiety and improve animal welfare (21), as daily supervision with opaque cages would be even harder under red light, changing the light system should not be one of the priorities for these institutions. On the contrary, it would be better to try to update the cage systems so that they allow unrestricted visualization, avoid physical lesions to the animals, and reduce the possibility of animals escaping.

Concerning the microbiological monitoring of colonies, it is interesting to remark that there is one diagnostic laboratory in Argentina, the Laboratory of Experimental Animals (LAE) (Faculty of Veterinary Sciences, National University of La Plata) that follows FELASA recommendations for the health monitoring of rodents in breeding colonies (13). According to the responses obtained, several facilities screen an incomplete set of microbiological agents. More importantly, 28% of the facilities report that they do not screen against any kind of pathogen, which have implications not only in terms of scientific rigor or animal welfare, but also in the health of the personnel that is potentially at risk of developing zoonosis. The extended use of antiparasitics, both preventively and therapeutically, could be a reflection of this faulty system. Despite this, reported health conditions were relatively infrequent (all but three clinical signs were reported in four or fewer facilities). Two of these three frequent conditions are highly related, as a recent article describes that cannibalism is actually a consequence of perinatal mortality (22). Although we cannot corroborate that this phenomenon is happening with our current data, it might explain the co-occurrence of these undesirable conditions in many facilities. The remaining frequent condition (barbering)

has been described as a behavioral problem arising from rearing in standard cages (23).

Supervision of the animals at appropriate intervals is key to guaranteeing their wellbeing (24). Unfortunately, supervision is suboptimal in the surveyed establishments due to the extensive incorporation of opaque cages and the absence of attending personnel during the weekends in 40% of the institutions. One positive finding of this questionnaire is that all institutions mention at least one source of training within their personnel. In this regard, despite the fact that in-house training is very extended (20 out of 25 institutions), all facilities also describe that they outsource their training to other institutions. In addition, some of this training is highly specialized, consisting of veterinarians and people with Laboratory Animal Technologist (LATG) degrees. This is remarkable since there is currently no legislation in the country regulating minimum requirements for the people working with laboratory animals. We believe that this could be ascribed to a long tradition of researchers, technicians, and educators in the field of Laboratory Animal Science (5). Indeed, Argentina is the only country in the region that has a 3-years undergraduate degree for LATG (Técnico Universitario para Bioterios) at the University of Buenos Aires. In this sense, the low rate of incidents with the animals (no biting reported in any of the facilities) is most likely a reflection of the preparation of the people working with them.

Cage fighting is currently one of the primary threats to mice welfare (16), a problem that has also been detected in this survey as several institutions reported that they heard vocalizations compatible with cage fighting. Nevertheless, this number is relatively low (just five reports) and only 32% of the animal facilities describe that they had to separate groups that were maintained stable after weaning in the last month. In the aforementioned article, the authors have identified individually ventilated cages and corncob bedding as the greatest predictors for fighting in the mouse cages (16), two components that are rare among Argentine institutions. Therefore, it would be interesting to study the epidemiology of cage fighting and confirm if the prevalence of agonistic behavior and lesions is compatible with the prevalence reported by American and European institutions (16, 25). However, it should be noted that single-housing is still a relatively common practice in the country, and can be one of the reasons for keeping in-cage aggression low.

All but two institutions provide some kind of environmental enrichment, with nest material being by far the most popular resource. This is unsurprising since it is highly preferred by mice (26) and has widely known benefits such as the reduction of male fighting (27), the improvement of breeding productivity (28), and the reduction of cold stress (29). The absence of adverse effects due to environmental enrichment could be ascribed to the fact that nest materials have virtually no detrimental effects (30). Interestingly, some facilities report varying the type of object throughout the year, which can help to reduce animal boredom (31). Nevertheless, it should be mentioned that this is purely an empirical practice, and we are not aware of previous research that standardizes or validates this procedure.

Uptake of non-aversive handling is still relatively low in the country, with fewer than half of the facilities reporting the use of these methods. Moreover, when employed, it was always combined with tail manipulation. In contrast, a recent survey about non-aversive handling with the majority of the participants from Europe and North America has described that 61% regularly use non-aversive handling (with 35% responding that they use it exclusively and 43% in combination with tail handling) (32). Still, it is important to note that dissemination campaigns to promote the incorporation of non-aversive handling are non-existent in Argentina. The aforementioned survey has highlighted the fact that unfamiliarity with the techniques is one of the causes for not using them (32). Non-aversive handling has many benefits not only in terms of animal welfare and the quality of scientific research (33–35), but also in the performance of the breeding colonies: breeding pairs handled with a tunnel produce, on average, one additional pup at weaning than mice handled by the tail (36). Therefore, a good strategy would be the dissemination of these methods to the scientific community by either the local/regional Laboratory Animals Science Associations or the different Scientific Bodies.

To sum up, the areas in which we see that there is greater space for improvement are recent refinements that can have a direct impact on animal welfare, such as non-aversive handling. These can be improved relatively easily with training programs and modifying the established husbandry. Other structural shortcomings/weaknesses will be harder to address, as they will require the commitment of the politicians to implement specific legislation, the establishment of suppliers that guarantee minimum standards, and the improvement of budgets to invest in animal facilities. All in all, the greatest strength of Argentine animal facilities is in the people that care for the animals on a day-to-day basis, in the large network of ethics committees that oversee animal research, and in the existence of the national record of animal facilities. Although adherence to this registry is voluntary, it is currently the only entity that allows for any kind of networking or action at the institutional level such as the execution of the present work.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

AR and SD conceptualized the idea and designed the survey. AR compiled the survey online and collected the answers. SD summarized the results and both authors jointly wrote

the manuscript. All authors approved the final version of the manuscript.

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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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2022.1031976/full#supplementary-material

References

1. Lewejohann L, Schwabe K, Häger C, Jirkof P. Impulse for animal welfare outside the experiment. *Lab Anim.* (2020) 54:150–8. doi: 10.1177/0023677219891754

2. National Research Council (NRC). *Guide for the Care and Use of Laboratory Animals, 8th edn*. Committee for the Update of the Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research. Available online at: https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf (accessed September 30, 2022).

3. Bayne K, Morris TH, France MP. Legislation and oversight of the conduct of research using animals: a global overview. In: Hubrecht R, Kirkwood J, editors. *The UFAW Handbook on the Care and Management of Laboratory and Other Research Animals*. Oxford: Wiley-Blackwell (2010). p. 107–23. doi: 10.1002/9781444318777.ch8

4. Rivera E, Hernández R, Carissimi AS, Pekow C. Laboratory animal legislation in Latin America. *ILAR J.* (2016) 57:293–300. doi: 10.1093/ilar/ilw017

5. Diaz SL. LAS around the globe. Argentina and Latin America: building slowly from a solid foundation. *Lab Anim.* (2022). 00236772221096527. doi: 10.1177/00236772221096527. [Epub ahead of print].

6. European Commission. *Report from the Commission to the European Parliament and the Council: 2019 Report on the Statistics on the Use of Animals for Scientific Purposes in the Member States of the European Union in 2015–2017*. Available online at: https://op.europa.eu/s/wQUv (accessed August 28, 2022).

7. Broom DM. Animal welfare science: history and concepts. In: Broom DM, editor. *Sentience and animal welfare*. Wallingford: CABI. (2014). p. 22–36. doi: 10.1079/9781780644035.0022

8. Fraser D. How Do the different measures relate to each other? In: Fraser D, editor. *Understanding Animal Welfare: The Science in its Cultural Context*. Oxford, UK: Wiley-Blackwell (2008). 324 p.

9. Mellor DJ, Reid CSW. Concepts of animal well-being and predicting the impact of procedures on experimental animals. In: Baker R, Jenkin G, Mellor DJ, editors. *Improving the Well-Being of Animals in the Research Environment*. Glen Osmond, SA: Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART) (1994). p. 3–18.

10. Mellor DJ, Beausoleil NJ, Littlewood KE, McLean AN, McGreevy PD, Jones B, et al. The 2020 five domains model: including human-animal interactions in assessments of animal welfare. *Animals.* (2020) 10:1870. doi: 10.3390/ani10101870

11. Resasco A, MacLellan A, Ayala MA, Kitchenham L, Edwards AM, Lam S, et al. Cancer blues? A promising judgment bias task indicates pessimism in nude mice with tumors. *Physiol Behav.* (2021) 238:113465. doi: 10.1016/j.physbeh.2021.113465

12. Ullman-Culleré MH, Foltz CJ. Body condition scoring: a rapid and accurate method for assessing health status in mice. *Lab Anim Sci.* (1999) 49:319–23.

13. Mähler M, Berard M, Feinstein R, Gallagher A, Illgen-Wilcke B, Pritchett-Corning K, et al. recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit colonies in breeding and experimental units. *Lab Anim.* (2014) 48:178–92. doi: 10.1177/0023677213516312

14. Diaz, SL. (2018) *Análisis de la situación de la ciencia y la tecnología de animales de laboratorio en Argentina. Elementos estratégicos para delinear una política en el área* (dissertation/master's thesis). Buenos Aires: Universidad de Buenos Aires.

15. David JM, Knowles S, Lamkin DM, Stout DB. Individually ventilated cages impose cold stress on laboratory mice: a source of systemic experimental variability. *J Am Assoc Lab Anim.* (2013) 52:738–44.

16. Theil JH, Ahloy-Dallaire J, Weber EM, Gaskill BN, Pritchett-Corning KR, Felt SA, et al. The epidemiology of fighting in group-housed laboratory mice. *Sci Rep.* (2020) 10:16649. doi: 10.1038/s41598-020-73620-0

17. Sakaguchi M, Inouye S, Miyazawa H, Kamimura H, Kimura M, Yamazaki S. Evaluation of countermeasures for reduction of mouse airborne allergens. *Lab Anim Sci.* (1990) 40:613–5.

18. Perkins SE, Lipman NS. Characterization and quantifiation of microenvironmental contaminants in isolator cages with a variety of contact beddings. *Contemp Top Lab Anim Sci.* (1995) 34:93–8.

19. Krohn TC, Hansen AK. Evaluation of corncob as bedding for rodents. *Scand. J. Lab Anim.* (2008). 35:231–6.

20. Castelhano-Carlos MJ, Baumans V. The impact of light, noise, cage cleaning and in-house transport on welfare and stress of laboratory rats. *Lab Anim.* (2009) 43:311–27. doi: 10.1258/la.2009.0080098

21. Hawkins P, Golledge HD. The 9 to 5 Rodent– Time for change? Scientific and animal welfare implications of circadian and light effects on laboratory mice and rats. *J Neurosci Methods.* (2018) 300:20–5. doi: 10.1016/j.jneumeth.2017.05.014

22. Brajon S, Morello GM, Capas-Peneda S, Hultgren J, Gilbert C, Olsson A. All the pups we cannot see: cannibalism masks perinatal death in laboratory mouse breeding but infanticide is rare. *Animals.* (2021) 11:2327. doi: 10.3390/ani11082327

23. Bechard A, Meagher R, Mason G. Environmental enrichment reduces the likelihood of alopecia in adult C57BL/6J mice. *J Am Assoc Lab Anim Sci.* (2011) 50:171–4.

24. National Research Council. *Guide for the Care and Use of Laboratory Animals*. Washington, DC: National Academies Press (2011). 220 p.

25. Lidster K, Owen K, Browne WJ, Prescott MJ. Cage aggression in group-housed laboratory male mice: an international data crowdsourcing project. *Sci Rep.* (2019) 9:15211. doi: 10.1038/s41598-019-51674-z

26. Van de Weerd HA, Van Loo PLP, Van Zutphen LFM, Koolhaas JM, Baumans V. Strength of preference for nesting material as environmental enrichment for laboratory mice. *Appl Anim Behav Sci.* (1998) 55:369–82. doi: 10.1016/S0168-1591(97)00043-9

27. Van Loo PLP, Van Zutphen LFM, Baumans V. Male management: coping with aggression problems in male laboratory mice. *Lab Anim.* (2003) 37:300–13. doi: 10.1258/002367703322389870

28. Gaskill BN, Winnicker C, Garner JP, Pritchett-Corning KR. The naked truth: breeding performance in nude mice with and without nesting material. *Appl Anim Behav Sci.* (2013) 143:110–6. doi: 10.1016/j.applanim.2012.10.009

29. Gaskill BN, Gordon CJ, Pajor EA, Lucas JR, Davis JK, Garner JP. Impact of nesting material on mouse body temperature and physiology. *Physiol Behav.* (2013) 110:87–95. doi: 10.1016/j.physbeh.2012.12.018

30. Van de Weerd HA, Van Loo PLP, Van Zutphen LFM, Koolhaas JM, Baumans V. Nesting material as environmental enrichment has no adverse effects on behavior and physiology of laboratory mice. *Physiol Behav.* (1997) 62:1019–28. doi: 10.1016/S0031-9384(97)00232-1

31. Meagher R. Is boredom an animal welfare concern? *Anim Welfare.* (2018) 28:21–32. doi: 10.7120/09627286.28.1.021

32. Henderson LJ, Smulders TV, Roughan JV. Identifying obstacles preventing the uptake of tunnel handling methods for laboratory mice: an international thematic survey. *PLoS ONE.* (2020) 15:e0231454. doi: 10.1371/journal.pone.0231454

33. Hurst JL, West RS. Taming anxiety in laboratory mice. *Nat Methods.* (2010) 7:825–6. doi: 10.1038/nmeth.1500

34. Clarkson JM, Dwyer DM, Flecknell PA, Leach MC, Rowe C. Handling method alters the hedonic value of reward in laboratory mice. *Sci Rep.* (2018) 8:1–8. doi: 10.1038/s41598-018-20716-3

35. Gouveia K, Hurst JL. Optimising reliability of mouse performance in behavioural testing: the major role of non-aversive handling. *Sci Rep.* (2017) 7:44999. doi: 10.1038/srep44999

36. Hull MA, Reynolds PS, Nunamaker EA. Effects of non-aversive versus tail-lift handling on breeding productivity in a C57BL/6J mouse colony. *PLoS ONE.* (2022) 17:e0263192. doi: 10.1371/journal.pone.0263192

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Nigerian indigenous hens show more discomfort-related behavior with visual separation than physical separation from their chicks: An exploratory study

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The Nigerian indigenous hens exhibit their full natural behavior repertoires, including maternal care. The strong maternal bond between the hen and her chicks is established prior to hatching. Maternal care of chickens is essential for both exotic and indigenous chickens. This study compared the behaviors of six hen-chick pairs in a physical (PHY) and visual (VIS) separation test for 10 min. All the six hen-chick pairs were subjected to PHY separation on the 8^{th} day of post-hatch and a VIS separation on the 12^{th} day of post-hatch. The PHY separation involved the use of a wire mesh to separate the hen from her chicks, while the VIS separation involved the use of a trampoline to separate the hen from her chicks. The hen's behavior was recorded during the 10-min separation period. Behaviors recorded included sitting, body shaking, pecking, movements toward the chicks, jumping, pacing, defecation, movements away from the chicks, and preening. We further grouped these nine behaviors into two categories: discomfort-related (pacing, movement toward chicks, body shaking, defecation, and jumping) and comfort-related (sitting, pecking, preening, and movement away from the chicks) behaviors. Before and after each separation, the hens were gently restrained, and a drop of blood was sampled from the wing vein to determine the blood glucose level. Their heart rate and eye temperature were also measured. A two-related samples test (Wilcoxon) was used to compare the behavior of the hens when subjected to the PHY and VIS separation. Eight out of the nine behaviors monitored did not differ between the separation types. However, the frequency of pacing by the hens was greater ($z = -2.201$, $P = 0.028$) in the VIS separation than in the PHY separation. Also, discomfort-related behavior was greater ($t_{(5)} = -2.717$, $P = 0.042$) during the VIS separation than the PHY separation. Comfort-related

behavior did not differ between the separation types. The change in eye temperature, heart rate, and blood glucose was similar in the two separation types. In conclusion, Nigerian indigenous hens displayed more discomfort-related behavior to the VIS separation from their chicks, but this was not associated with physiological responses indicative of stress.

KEYWORDS

behavior, maternal care, Nigerian indigenous chickens, separation types, welfare, pacing

Introduction

The Nigerian indigenous chicken is the most common poultry species found in the rural areas of Nigeria (1). They are commonly reared under an extensive or semi-intensive system (2). These chickens are raised for cultural and socio-economic purposes. The birds are usually provided minimal nutrition, medication, and shelter, which can compromise their welfare. In a scavenging system, the hen and her chicks may be separated temporarily (short-term) or permanently (long-term). During scavenging, a predator may kill the chicks, chicks may get lost when trapped by weeds, ropes, or threads, and a physical barrier such as a fence may not allow the hen to find her chicks. Also, the owner of the chickens may decide to wean the chicks early and sell them for financial reasons. The consequences of these circumstances on the welfare of the hens and the chicks are unknown but there is evidence that hens respond to their chicks in distress. Mother hens showed context-dependent behavioral responses. They also showed physiological responses such as changes in eye and comb temperature (a measure of stress-induced hyperthermia), heart rate, and heart rate variability (a measure of the activation of the sympathetic and parasympathetic nervous systems, respectively) when physically separated from their chicks. When the chicks are separated without any aversive stimulus, the hen showed no physiological changes in terms of heart rate and eye temperature but the mother hen responded to her chicks being puffed with a drop in eye temperature and an increase in heart rate (3).

The welfare of an animal is good when the animal is allowed to perform its natural behaviors (4). Although not all natural behaviors are beneficial, maternal behavior is one of the most important natural behaviors of the indigenous chickens which has helped them survive and maintain their population for several years. Maternal behavior in chickens includes nesting, egg-laying, brooding, and post-hatch care of chicks (5). Broodiness is often considered an uneconomical trait because the hen stops laying (6). This trait has been selected against in most commercial laying hens. However, most indigenous breeds remain genetically unselected for increased egg production and still exhibit broodiness, making it possible for them to incubate their eggs and hatch their chicks by themselves. The Nigerian indigenous hens spend 88–93% of their time sitting on the eggs and 0.06–0.11% on feeding and drinking during the brooding period (7). Broodiness is also associated with changes in breast temperature and blood glucose levels in Nigerian indigenous hens (7). In a comparative study, the Yoruba ecotype of the Nigerian indigenous hens spent more time sitting on the eggs during brooding than the Fulani ecotype hens (8).

Bonding in chickens starts a few days before hatching, at a time when the hen and the developing embryo begin to communicate through vocalization (9). This pre-hatching communication enables the chicks to recognize the voice of their mothers after hatching. After hatching, the hen serves as the role model for her chicks by providing warmth and protection. The hen teaches her chicks how and where to forage and escape from predators using a variety of calls (9). The Thai native hens protect their chicks by being vigilant, aggressive, and emitting an alarm call (10). Maternal care of chickens is essential and cannot be underestimated. Also, maternal deprivation of chicks has welfare consequences (9). Chicks reared without mothers are highly fearful, aggressive, and displayed higher feather pecking and cannibalism (11–15).

Chickens are a precocial species, which means that the chicks can survive on their own in an artificial environment after hatching. However, in the natural environment, day-old chicks cannot survive without their mother's care because they cannot regulate their body temperature and escape from predators. The external temperature experienced by red junglefowl chicks in the wild ranges from 19–28°C, a temperature range that the chicks may find difficult to cope with until they are about 10 days old (16). Prior to this age, the mother hen provides the needed warmth (16).

Mother hens permanently separated from their chicks after 5 to 10 days post-hatch, resumed egg-laying earlier, but the chicks had a low survival rate (6). Although Amin et al. (6) did not report the behavior of the hens and chicks during the separation, the abrupt weaning could be stressful for both the hen and her chicks. Indigenous chickens are mostly reared by rural farmers in most developing countries. The hens are used to produce the next generation of the chicken flock by allowing them to incubate and care for the chicks. There seems to be

no information on the best approach to artificially wean chicks from the hens. Some farmers may be patient enough for the hen to wean the chicks naturally (which could range from 5–12 weeks post-hatch), but farmers that use this hen as a "natural hatcher" prefer to wean the chicks immediately after hatching and place another set of eggs under the hen to incubate for another 21 days. Using the broody hen to hatch two batches of chicks consecutively may have a detrimental effect on the hen's welfare and need further investigation. Also, there is a need to develop a more welfare-friendly method of weaning chicks from their mothers than the conventional sudden separation.

After hatching, hens display maternal aggression, which is necessary to protect their chicks from environmental threats such as humans and predators. The survivability of the chicks in the natural environment is highly dependent on how protective the mother hen is. Since these chickens are reared under scavenging systems, it is of utmost importance to identify and select hens with a high level of maternal aggression, as this would ensure better chick survivability. So, in this exploratory study, we compared the behaviors of six Nigerian indigenous hens when separated physically or visually from their chicks. The use of less invasive means of assessing stress, such as infra-red thermography and heart rate monitors, serves as a refinement of the procedures for assessing animal welfare. Glucocorticoids are known to be stress hormones, but they have several limitations, some of which are the need to sample blood within a short time (<3 mins), handling the animal can trigger the release of glucocorticoids and an increase in glucocorticoids does not indicate whether the subject is experiencing a positive or negative valence (17). We hypothesized that hens would find the two separation types different and therefore display discomfort-related behaviors coupled with stress responses to the separation type that prevents them from having more contact with their chicks. The implications of these separation types are discussed with respect to the management and animal welfare purposes.

Materials and methods

Ethical statement

The procedure for the experiment was approved by the Animal Care and Use Committee of the College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. All birds used in the experiment were provided with proper care and management, and were not exposed to unnecessary discomfort.

Experimental site

The experiment was conducted at the Poultry Unit of the Federal University of Agriculture, Abeokuta, Nigeria. The experimental site lies on latitude 7°10'N and longitude 3°2'E. It is located 76 m above sea level, in the tropical rainforest vegetation zone, and has a mean temperature of 28.5°C.

Experimental procedure

The birds used in this study were sourced from an existing flock of sexually mature Nigerian indigenous chickens (30 hens and three cocks) of the Yoruba ecotype. The average weight of the cocks and hens was 1250 ± 90.5 g and 850 ± 50.5 g, respectively. The birds were housed in deep litter pens with a mating ratio of 10 hens to one cock per pen (975g/m^2). The birds were fed layer mash (16.5% CP, 2725–2980 Kcal/kg metabolizable energy, 5% fat/oil, 6% crude fiber, 3.60% calcium, 0.45% available phosphorus, 0.80% lysine, 0.34% calcium, and 0.30% salt) at the rate of 120 g/bird/day (the recommended quantity for a laying hen to prevent fat accumulation that can affect laying) and fresh water was provided *ad libitum*. The pens were furnished with wooden ladder perches (each consisting of three tiers; the lower tier at 20 cm, the middle tier at 50 cm, and the upper tier at 90 cm above the ground) for the birds to roost at night and nest boxes for the hens to perform their natural egg-laying behavior. The health of the birds was checked daily. Eggs laid in the nest boxes were left to encourage broodiness. Any hen that became broody (as demonstrated by continuous sitting on the eggs for three consecutive days) was separated into a brooding pen and provided with 10 fresh eggs (eggs picked from the remaining flock) to incubate and feed and water *ad libitum*. The expected hatching date from the day the hen was separated and provided with the fresh eggs to incubate was noted for each hen. The poultry house was open-sided, so birds were exposed to natural daylight (~12L:12D) and daily fluctuations in temperature and humidity.

We intended to have data from as many hens that became broody as possible. However, there were cases of two broody hens for which we could not get data. The first hen left its nest on the 17th day of brooding and did not return to sit on the eggs. We later found out that none of the eggs was fertile. The hen was probably sensitive enough to detect this, as the hen must have expected that there should be a pre-hatching communication between her and the developing embryos at that stage of incubation. The second hen died during brooding; the cause was unknown to us.

The six mother hen-chick pairs were undisturbed for the first 7 days post-hatch. Water was provided in a bell drinker (diameter = 21.5 cm, depth = 20.3 cm) and feed was provided in round plastic tray feeders (diameter = 20.0 cm). Chick mash (21% CP, 3000 Kcal/kg metabolizable energy) was provided to the hen and her chicks after hatching (as it was not possible to feed them separately). All six hen-chick pairs (each hen-chick pair served as a replicate) experienced both separation types; physical separation on day 8 and visual separation on day 12

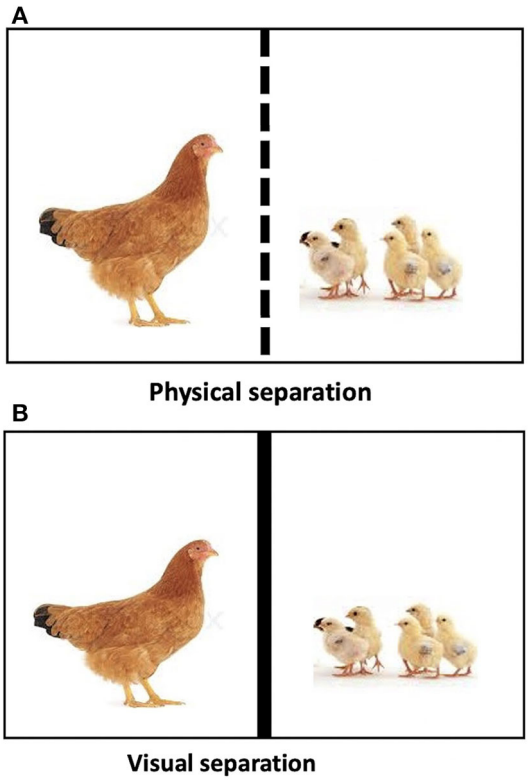

FIGURE 1
(A) Hen-chicks' pair subjected to physical separation in a test arena. **(B)** Hen-chicks' pair subjected to visual separation in a test arena.

post-hatch. Each hen-chick pair was tested once on each of these days for a 10-min period.

Physical separation test

On the 8th day of post-hatch, each hen-chick pair was placed in a test arena separated by a wire mesh partition, which allowed visual and auditory contact between the mother hen and her chicks (Figure 1A). The hen's behaviors were recorded using a digital camera (Fujifilm S2950, made in China) during a 10-min physical separation. The frequency of behaviors, such as movements away from chicks (AFC), movements toward the chicks (TC), defecation, preening, sitting, body shaking, pecking, jumping, and pacing was recorded. A description of the behaviors is presented in Table 1.

Visual separation test

On the 12th day of post-hatch, the same test arena used for the physical separation test was used, but instead of having a wire mesh separating the hen from her chicks as in the physical separation, a purple trampoline was securely attached to the wire mesh partition between the hen and her chicks, thus allowing only auditory but no visual contact between the mother hen and her chicks (Figure 1B). The visual separation lasted for 10 min, and the behaviors of the hens were recorded.

The nine behaviors were further grouped into two categories: discomfort-related (pacing, movement toward chicks, body shaking, defecation, and jumping) and comfort-related (sitting, pecking, preening, and movement away from the chicks) behaviors.

TABLE 1 Ethogram of mother hen behavior monitored during visual and physical separation.

Behavioral category	Description
Movement away from chicks (AFC)	The hen moves away from the barrier between her and her chicks
Movement toward chicks (TC)	The hen moves closer to the barrier between her and her chicks
Defecation	Excretion of feces by the hen in the test arena
Preening	The hen uses its beak to arrange its feathers
Sitting	Hen lying down on her chest in the test arena
Body shaking	The hen shakes and ruffles her feathers
Pecking	The hen pecking on wooden materials in the test arena
Jumping	The hen jumps to escape from the test arena to reunite with her chicks
Pacing	The hen moving to and fro in the test arena without rest

Physiological data collection

Before and after each separation type, physiological parameters such as heart rate, eye temperature, and blood glucose level were measured. The heart rate of the hens was measured with a stethoscope placed on the chest region of the hen and the number of beats per 15 s was counted and multiplied by four to give the number of beats per minute. The eye temperature of the hens was measured using an infrared thermometer (Model: IT-122, accuracy $\pm$ 0.2°C, made in China) pointed about 2 cm away from the eye of the hen. Finally, a drop of blood was sampled from the wing vein onto a glucose strip, which was immediately inserted into an ACCU-CHEK active glucose meter (manufactured by ROCHE Mannheim, Germany) to determine the blood glucose level.

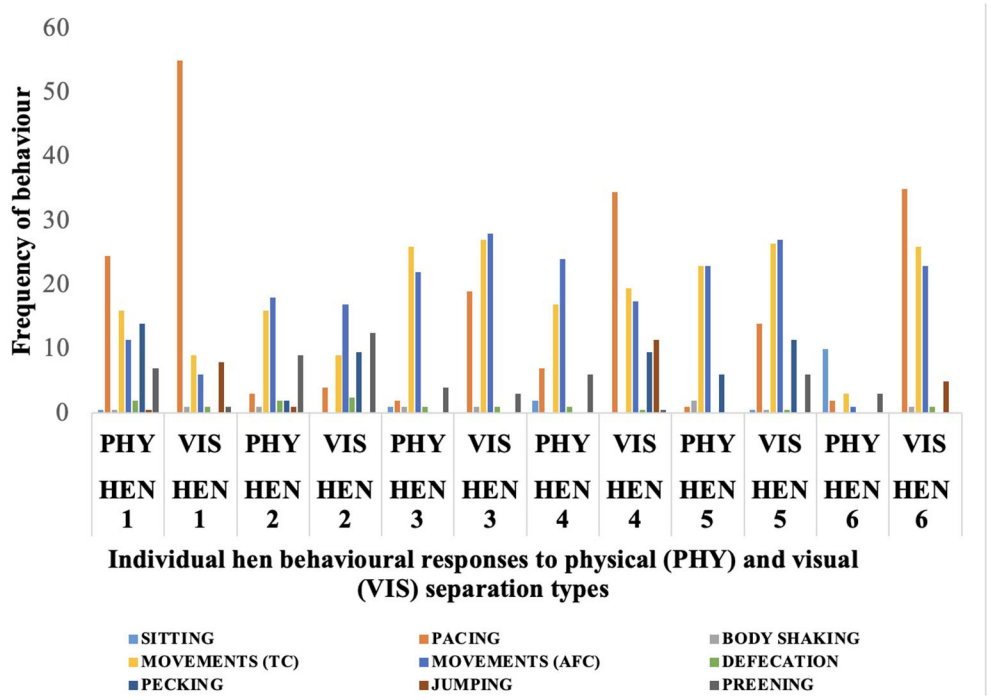

FIGURE 2
Frequency of behaviors exhibited by each of the six hens during the physical (PHY) and visual (VIS) separation tests for 10-minutes from their chicks. 1-6 represent the individual hen numbers. TC, toward chicks; AFC, away from chicks.

These physiological parameters were taken within 2 min of restraining the hen.

Statistical analysis

The individual differences between the six hen-chick pairs during the two separation types (physical test on day 8 and visual test on day 12 post-hatch) were analyzed using descriptive statistics (bar charts). We compared each of the nine behaviors monitored from the hens in these two separation types using a two-related samples test (Wilcoxon). Similarly, discomfort-related and comfort-related behaviors displayed by each hen in the two separation types were compared using paired sample *t*-test. The change (after separation minus before separation) in the eye temperature, heart rate, and blood glucose of the six hens during the two separation types were also compared using a paired sample *t*-test. All statistical procedures were undertaken using the IBM SPSS statistical software (Version 23).

Results

Behavioral responses of individual hens to the physical and visual separation from their chicks are shown in Figure 2. Some hens had extremely low or high values compared with the mean for some behaviors. During the physical separation, Hen 6 showed a very low movement frequency of 3 toward her chicks compared with the mean value of 17 shown by the other hens, and this same hen displayed a sitting frequency of 10 compared with the mean of other hens that sat down just once. Pacing frequency during the visual separation also varied substantially, with Hen 1 pacing more than 50 times while Hen 4 and 6 paced about 35 times. During visual separation, the frequency of sitting behavior was very low. Hen 5 sat only once during visual separation, while the other five hens did not sit at all during the 10-min period. These variations are evident as outliers in the box plots in Figures 3–7.

There was no difference in the frequencies of eight out of the nine behavior between the two separation types; the movement of the hen toward her chicks ($z = -0.315$, $P = 0.752$, Figure 3A), movement away from her chicks ($z = -0.314$, $P = 0.753$, Figure 3B), preening ($z = -0.420$, $P = 0.674$, Figure 4A), body shaking ($z = -0.552$, $P = 0.581$, Figure 4B), defecation ($z = -0.276$, $P = 0.783$, Figure 5A), sitting ($z = -1.625$, $P = 0.104$, Figure 5B), pecking ($z = -0.365$, $P = 0.715$, Figure 6A), and jumping ($z = -1.461$, $P = 0.144$, Figure 6B). However, the frequency of pacing ($z = -2.201$, $P = 0.028$, Figure 7) was greater when the hens were separated from their chicks visually than when they were separated physically.

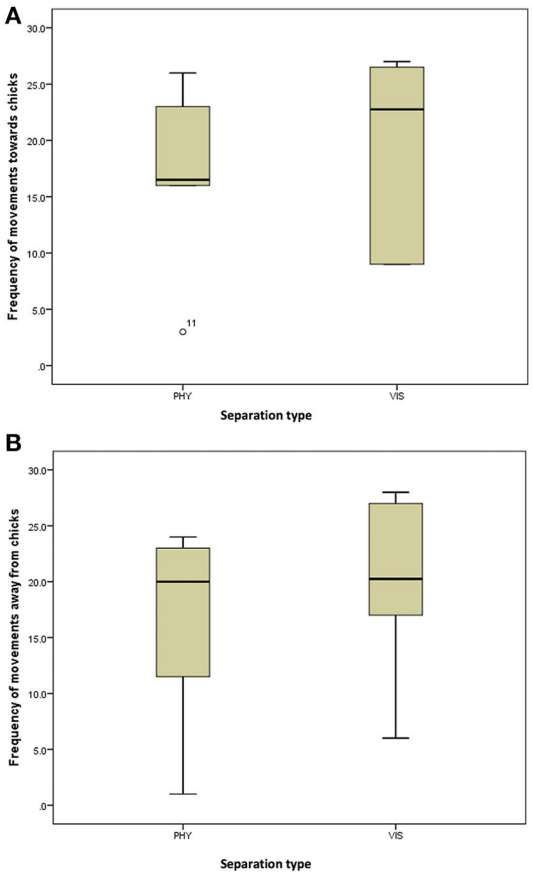

FIGURE 3
(A) Frequency of movement of the mother hens toward their chicks when subjected to 10-min physical (PHY) or visual (VIS) separation. **(B)** Frequency of movement of the mother hens away from their chicks when subjected to 10-min physical (PHY) or visual (VIS) separation.

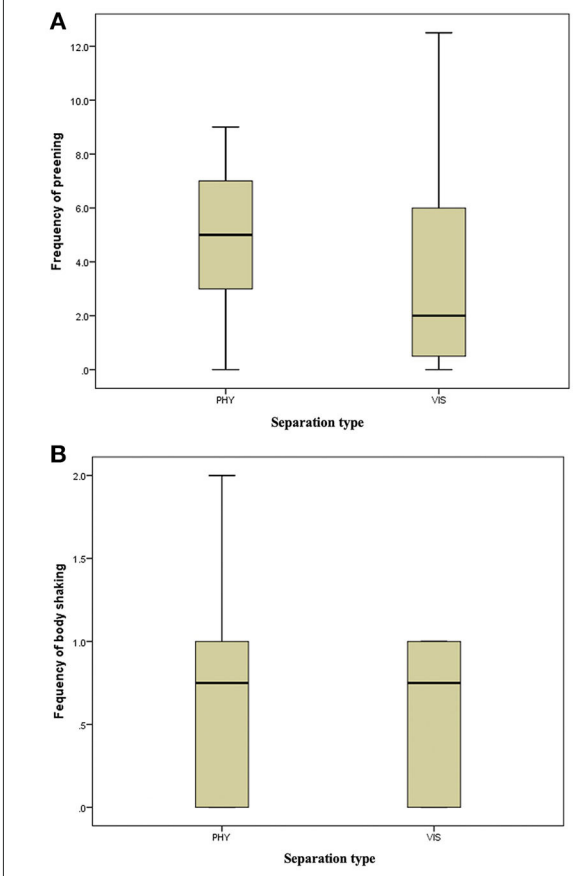

FIGURE 4
(A) Frequency of preening of the mother hens when subjected to 10-min physical (PHY) or visual (VIS) separation from their chicks. **(B)** Frequency of body shaking of the mother hens when subjected to 10-min physical (PHY) or visual (VIS) separation from their chicks.

For the grouping of the behaviors, there was a greater $[t_{(5)} = -2.717, P = 0.042]$ display of discomfort-related behavior in hens during visual separation than in physical separation, but no difference $[t_{(5)} = -0.231, P = 0.827]$ in comfort-related behavior in hens subjected to physical or visual separation (Figure 8).

Finally, the actual values of the eye temperature, heart rate, and blood glucose before and after each separation and the changes (after separation minus before separation) in eye temperature, heart rate, and blood glucose were similar ($P > 0.05$) in both separation types (Table 2).

Discussion

The low number of hens in this study constrained us from making a definite conclusion, but we discuss the implications of these preliminary results for the management and welfare implications of the indigenous chickens. This exploratory study compared the behaviors of Nigerian indigenous hens when separated physically and visually from their chicks for 10 min on the 8th and 12th days of post-hatch, respectively. The tests on the six hen-chick pairs were conducted on different days to reduce the level of stress and the possibility of a masking effect of one separation type on the other one.

The experiment did not follow a cross-over design. Hence, the possibility of a confounding effect of the chicks' age with the type of separation cannot be totally ruled out. Nevertheless, there were only 3 days between the two tests and Edgar et al. (3) observed no effect of counterbalancing hens on their behavioral and physiological responses to their 3 to 4-week-old chicks' distress conditions.

These two separation methods were used because they were the most commonly occurring scenarios in the life of a scavenging mother hen and chicks. The separation lasted for 10 min to understand the hens' immediate behavior when

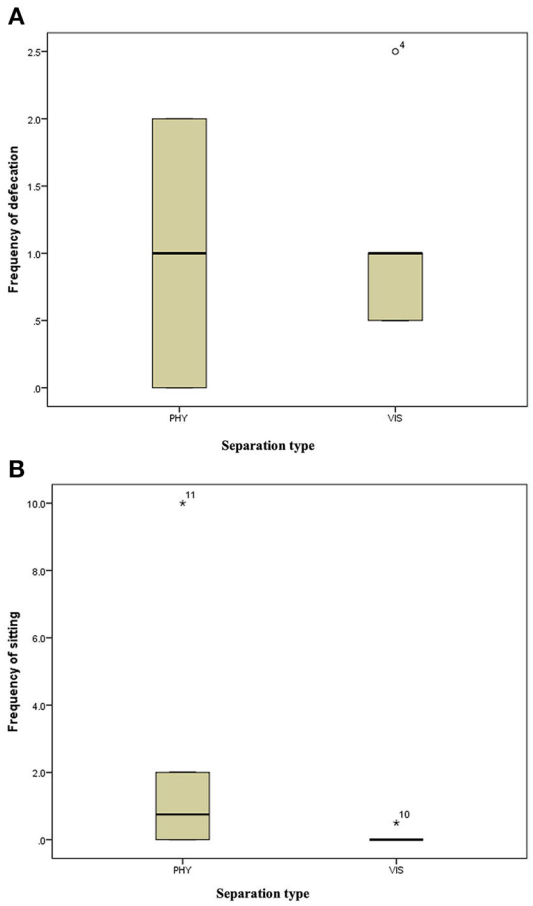

FIGURE 5
(A) Frequency of defecation of the mother hens when subjected to 10-min physical (PHY) or visual (VIS) separation from their chicks. **(B)** Frequency of sitting of the mother hens when subjected to 10-min physical (PHY) or visual (VIS) separation from their chicks.

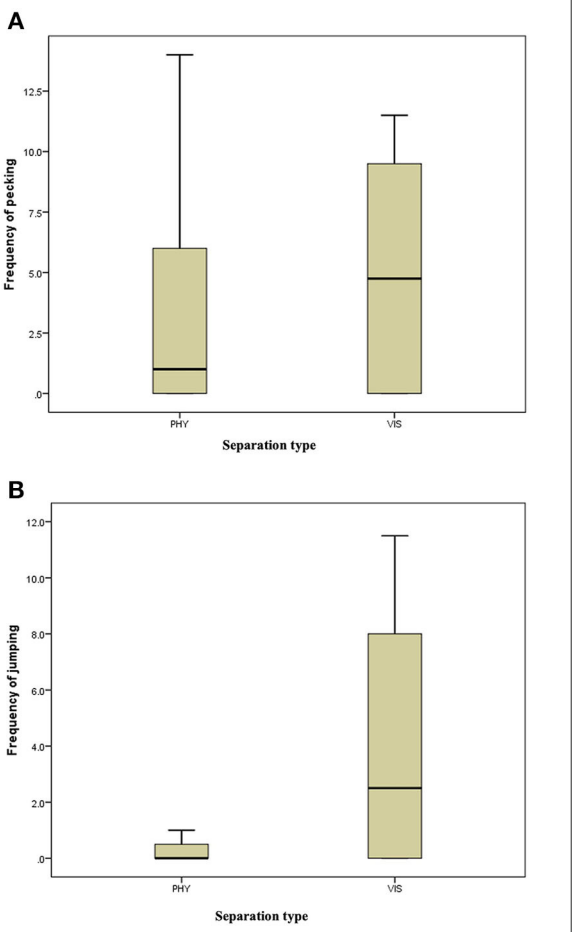

FIGURE 6
(A) Frequency of pecking of the mother hens when subjected to 10-minute physical (PHY) or visual (VIS) separation from their chicks. **(B)** Frequency of jumping of the mother hens when subjected to 10-minute physical (PHY) or visual (VIS) separation from their chicks.

separated from their chicks. We adopted a short-term separation based on previous reports that long-term (4-h) separation of 4-day-old chicks resulted in the chicks not being able to identify their mothers afterward (18). Also, the removal of 3-day-old chicks from their mothers caused a fast reduction in maternal responsiveness. The hen stopped clucking completely 4 days after withdrawal, but 56% of them still made tidbits calls, which decreased to 33% after a week of chick removal (19).

The low number of broody hens (20%) could be attributed to the adoption of a natural brooding method in this study, where eggs were left in the nest boxes and the hens were exposed to natural conditions (12L:12D). However, inducing broodiness by increasing the daylight to 16L in addition to the provision of eggs in the nest box resulted in 46.7% of brooding in the Silkie and Wyandotte hens (11, 20). It would be interesting to investigate if the induction of broodiness works in tropical breeds as reported in temperate breeds.

After hatching, hens become aggressive toward intruders to protect their chicks, a behavior known as maternal aggression. The intruders could be humans or predators. The mother hen displays maternal aggression for as long as the chicks are still under her care, especially in the first few weeks after hatching. The hens in the current study seemed to find the physical separation type less stressful, probably because they could see their chicks and communicate with them. Our result agrees with the report of Madec et al. (21) that hens experience less stress if they have visual contact with their chicks but could not come in contact with them physically. This type of separation could be adopted by rural poultry farmers as a means of a gradual weaning process instead of the abrupt weaning of chicks from their mothers. The farmers can subject the mother and chicks to physical separation a few times a day for several days before the chicks are finally weaned. By doing

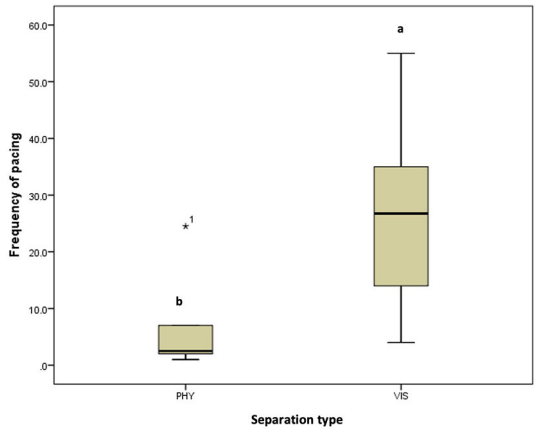

FIGURE 7
Frequency of pacing of the mother hens when subjected to 10-minute physical (PHY) or visual (VIS) separation from their chicks.[ab] Means differ at $P < 0.05$.

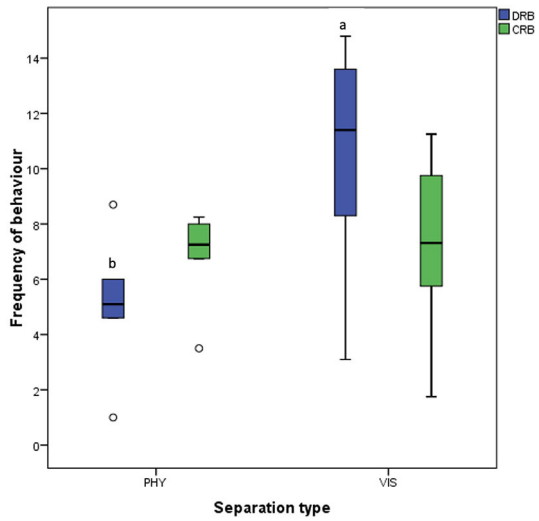

FIGURE 8
Frequency of discomfort-related (DRB) and comfort-related (CRB) behaviors of the mother hens when subjected to 10-minute physical (PHY) or visual (VIS) separation from their chicks. [ab] Means differ at $P < 0.05$ for discomfort-related behaviors.

this, the chicks would have gotten used to being separated from their mothers, and when finally weaned, they would not find the weaning process stressful. However, this proposed welfare-friendly weaning process of chicks requires investigation to determine how often, at what age, and how many days are required for the chicks to become accustomed to this process.

On the other hand, the mother hens displayed greater discomfort-related behaviors (pacing, movement toward the chicks, body shaking, defecation, and jumping) when visually separated from their chicks. This implies that the mother hens are more distressed by this type of separation because they could only communicate with their chicks but not see each other.

Some hens hatch a good number of chicks, but within the first week of life, most of the chicks have been predated upon, while some are able to keep all or most of their chicks for survival. Since these indigenous chickens are reared by poor rural farmers under a scavenging system, there is a need to identify and select breeds with high maternal care and aggression. For greater survivability of the chicks in the natural environment, the aggressiveness of the hens is a determining factor. For this study, we discovered some individual differences in the hens' behavior that could indicate their maternal styles.

A preliminary observation of the recorded behaviors of the hens was made and used to develop the ethogram reported in this study, as we could not find any existing ethogram for this kind of test in the literature. We observed some individual differences between the hens. The differences observed could be related to the differences in the number of chicks reared by each hen (the number of chicks ranged from three to seven in this study). There could be the possibility of maternal aggression corresponding with the hen's having a lower or greater number of chicks to protect. This would be a research idea for future studies. For instance, Hen 1 paced more than 50 times while Hens 4 and 6 paced about 35 times. It could be speculated that these three hens, which showed high pacing, might have a better mothering ability and were not ready to give up in their attempt to reunite with their chicks. Selection and multiplying hens with this trait could be beneficial for outdoor production because an increased maternal aggression confers on them the ability to protect their chicks when faced with real-time predators. Some mother hens go to the extreme of attacking predators such as hawks or snakes to protect their chicks (personal observation).

In the six hen-chicks pairs used in this study, eight of the behaviors monitored are performed in a similar pattern by hens in both separation types which implies that they reacted similarly to being separated from their chicks, irrespective of whether it is a physical or visual separation. However, the pacing frequency or frequency of discomfort-related behavior (pacing, movement toward chicks, jumping, defecation, and body shaking) is higher in hens during visual separation, which might suggest that the hens perceived the visual separation to be more stressful. During the visual separation, the hen and chicks could only communicate through vocalization. Increased pacing has been associated with restlessness. Animals tend to show an increased pacing when they are under stress or unable to express species-specific behaviors (22, 23). Pacing is considered a stereotypic behavior (24–26) when it is performed for no

TABLE 2 Physiological responses, actual values, and changes (after separation minus before separation) of Nigerian indigenous hens to physical and visual separation from their chicks.

	Eye temperature (°C)	Heart rate (beats/minute)	Blood glucose (g/dL)
Physical separation			
Before	37.27 ± 0.03	176.18 ± 0.17	203.88 ± 0.17
After	37.35 ± 0.25	176.52±3.03	188.88±6.02
Changes	0.08 ± 0.23	0.33 ± 3.07	−15.00 ± 5.98
Visual separation			
Before	37.53 ± 0.05	175.55 ± 0.73	185.40 ± 0.27
After	37.50 ± 0.33	174.38 ± 6.57	178.98 ± 5.05
Changes	−0.03 ± 0.31	−1.17 ± 6.60	−6.42 ± 4.90

Values are means ± SE.

apparent reason. The pacing behavior observed in this study is a way for the hen to express her discomfort at being separated visually, as she was looking for all means possible to reunite with her chicks.

There was the possibility that the hens were more stressed in the visual separation test. However, this was not supported by physiological responses. The reason for the lack of a difference in the stress responses between the physical and visual separation types could be attributed to the low number of hens used in this study or the short duration of the separation period (10-min). The choice of 10-min was intentional to reduce the distress experienced by the hens to the barest minimum.

One of the limitations of this study was that we failed to monitor the behavior and physiological responses of the chicks during the separation period. During the separation period, the chicks made distress calls in both separation types (personal observation). Wauters and Richard-Yris (27) also reported that chicks began to emit distress calls when they lost visual contact with their mothers (27). However, there may be a possibility of the chicks emitting a highly intense type of distress call depending on how stressful they might have perceived the two separation types. If the chicks perceive the visual separation to be more stressful, they could communicate this to their mothers, which might arouse her emotions to become more restless by increased pacing. Hens understand the distress conditions of their chicks (3). Mother hens modify their behaviors based on the signals from their chicks. In a recent study where chicks of two age categories (5–6 weeks old and 5–7 days old) were isolated from conspecifics for 5 min, the distress call made by the 5–7 days old chicks were of a greater peak frequency, and the vocalization characteristics in both chick groups were positively correlated with changes in surface body temperatures (28). Further studies are therefore required to investigate the effects of the two separation types on the chick's behavior and physiology.

Conclusion

This is an exploratory study on the behavior and physiological responses of Nigerian indigenous hens to visual and physical separations from their chicks. The behaviors of the hens are similar in the physical and visual separations, but hens reacted to visual separation from chicks by increasing pacing. Overall, hens displayed greater discomfort-related behaviors during visual separation than physical separation from their chicks. Physiological responses are similar in both separation types. Concrete conclusions cannot be made due to the low sample size used in this study. However, the findings from the study could serve as insights for future research on these chickens. Further studies should investigate the behavior, physiology, and distress call characteristics of the chicks under these two separation types. Future studies are needed to compare the responses of different chicken breeds or Nigerian indigenous chicken ecotypes to these separation types. Finally, it would be interesting to know whether maternal aggression has a positive influence on the chicks' survival and welfare.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The procedure for the experiment was approved by Animal Care and Use Committee of the College of Animal Science and Livestock Production of the Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

Author contributions

OI was involved in conceptualization, investigation, data collection, data analysis, and manuscript writing. SD and KO were involved in the investigation and manuscript writing. OPO, OEO, OF, and VO were involved in the investigation and data collection. All authors contributed to the article and approved the submitted version.

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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.

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References

1. RIM. *Nigerian National Livestock Resource Survey. Report by Resource Inventory and Management Limited (RIM) to Federal Department of Livestock and Pest Control Services (FDL&PCS)*. Abuja (1992) 432 p.

2. Ige AO, Salako AE, Yakubu A, Adeyemi SA. Qualitative traits characterization of Yoruba and Fulani ecotype indigenous chickens in derived savannah zone of Nigeria. *Int J Poult Sci.* (2012) 11:616–20. doi: 10.3923/ijps.2012.616.620

3. Edgar JL, Lowe CJ, Paul ES. Avian maternal response to chick distress. *Proceed Royal Soc of B: Biol Sci.* (2011) 278:3129–34. doi: 10.1098/rspb.2010.2701

4. FAWC. *Second Report on Priorities for Research and Development in Farm Animal Welfare*. Tolworth: MAFF (1992).

5. Chen SJ Li WB. Studies on the broodiness in Wumeng black-bone chicken. *Bullet Shanghai Husbandry Vet.* (2007) 5:84.

6. Amin MJR, Howlider MAR, Ali MA. Effects of chick separation and feeding on the performance of hens and chicks. *Bangladesh Veterinarian.* (2009) 26:13–6. doi: 10.3329/bvet.v26i1.4626

7. Iyasere OS, Ajayi OD, Alade SO, Akinbode VO. Behaviour, physiology, and body mass of Nigerian indigenous hens during brooding. *Agricultura Tropica et Subtropica*. (2019) 52:43–7. doi: 10.2478/ats-2019-0005

8. Oyeniran VJ, Iyasere OS, Durosaro SO, Fasasi FB, Odetayo PO, Ogunfuyi SA, et al. An exploratory study on the differences in maternal care between two ecotypes of Nigerian indigenous chicken hens. *Front Vet Sci Animal Behav Welfare Sec*. (2022) 9:980609. doi: 10.3389/fvets.2022.980609

9. Edgar J, Held S, Jones C, Troisi C. Influences of maternal care on chicken welfare. *Animal.* (2016) 6:2. doi: 10.3390/ani6010002

10. Nasinprom N, Nutthakornkul J, Nitipot P, Insee J. Observations on maternal behaviour of Thai native hens in the village chicken production systems. *Khon Kaen Agri J*. (2015) 43:2.

11. Rodenburg TB, Bolhuis JE, Koopmanschap RE, Ellen ED, Decuypere E. Maternal care and selection for low mortality affect post-stress corticosterone and peripheral serotonin in laying hens. *Physio Behav*. (2009) 98:519–23. doi: 10.1016/j.physbeh.2009.08.006

12. Shimmura T, Kamimura E, Azuma T, Kansaku N, Uetakek K, Tanaka T. Effect of broody hens on behavior of chicks. *Appl Anim Behav Sci.* (2010) 126:1125–133. doi: 10.1016/j.applanim.2010.06.011

13. Fält B. Differences in aggressiveness between brooded and non-brooded domestic domestic chicks. *Appl Anim Ethol.* (1978) 4:211–21. doi: 10.1016/0304-3762(78)90112-8

14. Rodenburg TB, Koene P, Spruijt BM. Reactions to frustration in high and low feather pecking lines of laying hens from commercial or semi-natural rearing conditions. *Behav Proc.* (2004) 65:179–88. doi: 10.1016/j.beproc.2003.09.003

15. Riber AB, Wichman A, Braastad BO, Forkman, B. Effects of broody hens on perch use, ground pecking, feather pecking and cannibalism in domestic fowl (Gallus gallus domestic). *Appl Anim Behav Sci.* (2007) 106:39–51. doi: 10.1016/j.applanim.2006.07.012

16. Sherry DF. Parental care and the development of thermoregulation in red junglefowl. *Behavior*. (1981) 76:250–79. doi: 10.1163/156853981X00103

17. Iyasere OS, Durosaro SO, Oyeniran VJ, Daramola JO. Is an increase of glucocorticoid concentrations related to the degree of arousal or valence experienced by an animal to a stimulus? *Domest Anim Endocrinol.* (2022) 81:106752. doi: 10.1016/j.domaniend.2022.106752

18. Kent JP. Experiments on the relationship between the hen and chick (*Gallus gallus*): the role of the auditory mode in recognition and the effects of maternal separation. *Behaviour*. (1987) 102:1–14. doi: 10.1163/156853986X00018

19. Leboucher G, Richard-Yris MA, Williams J, Chadwick A. Incubation and maternal behavior in domestic hens: influence of the presence of chicks on circulating luteinising hormone, prolactin and oestradiol and on behavior. *Br Poult Sci.* (1990) 31:851–62. doi: 10.1080/00071669008417316

20. Rodenburg TB, Uitdehaag KA, Ellen ED, Komen J. The effects of selection on low mortality and brooding by a mother hen on open-field response, feather pecking and cannibalism in laying hens. *Animal Welfare*. (2009) 18:427–32.

21. Madec I, Pageat P, Bougrat L, Saffray D, Falewe C, Gervasoni MA, et al. Influence of a semio-chemical analogue on growing performances and meat quality of broilers. *Poult Sci.* (2006) 87:222–5. doi: 10.1093/ps/85.12.2112

22. Mason GJ, Latham NR. Can't stop won't stop: is stereotypy a reliable animal welfare indicator? *Anim Welf.* (2004) 13:57–69.

23. Manteca X, Salas M. *Stereotypies as Animal Welfare Indicators*. Zoo Animal Welfare Fact Sheet (2015).

24. Lutz C, Well A, Novak M. Stereotypic and self-injurious behavior in rhesus macaques: a survey and retrospective analysis of environment and early experience. *Am J Primatol.* (2003) 60:1–15. doi: 10.1002/ajp.10075

25. Gottlieb DH, Capitanio JP, Mccowan B. Risk factors for stereotypic behavior and self - biting in rhesus macaques (Macaca mulatta): animal's history, current environment, and personality. *Am J Primatol.* (2013) 75:995–1008. doi: 10.1002/ajp.22161

26. Poirier C, Bateson M. Pacing stereotypies in laboratory rhesus macaques: implications for animal welfare and the validity of neuroscientific findings. *Neuroscience and Biobehavioural Reviews.* (2017) 83:508–15. doi: 10.1016/j.neubiorev.2017.09.010

27. Wauters AM, Richard-Yris MA. Mutual influence of the maternal hen's food calling and feeding behavior on the behavior of her chicks. *Dev Psychobiology.* (2002) 41:25–36. doi: 10.1002/dev.10042

28. Iyasere OS, Taylor, PS, Durosaro SO, Oyeniran VJ, Oyekunle OE, Ehighor TF. Nigerian indigenous chicks show age related difference in vocalization characteristics during short term isolation. *Proceedings of the 55th Congress of the International Society for Applied Ethology, theme 'Animal Behaviour and Beyond'* edited by Kjosevski M, Waiblinger S, Ilieski V. September 3-8, 2022 Ohrid, North Macedonia, pg 175.

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Animal welfare knowledge, attitudes, and practices among livestock holders in Ethiopia

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Improving animal welfare is a human responsibility and influenced by a person's values and experiences. Thus, it is critical to have an in-depth understanding of the knowledge, attitudes, and practices (KAP) of animal welfare among animal owners. For livestock in Ethiopia, the greatest proportion of livestock are reared by pastoral and mixed crop-livestock communities. A cross-sectional survey covering a range of species and animal welfare aspects was carried out on a total of 197 household (117 pastoral and 80 crop-livestock owners) and recorded information on 34 animal welfare KAP items. Item response theory models (IRT) were fitted to the data from KAP items to estimate the probability of correctly answering an item. This was used as a function of the respondents' KAP level. Overall, the highest percentage of desirable scores was recorded for the knowledge scale (35.7%) and the lowest was for the practice scale (24.6%). A significant correlation ($P < 0.01$) was found between knowledge of the farmers and their attitude toward animal welfare and self-reported practices. Generally, households practicing mixed crop-livestock farming system had better animal welfare knowledge, attitude, and practice than pastoralist. Mixed crop-livestock farmers had better knowledge on items related to observing the nutrition condition of the animal, animal-human relationship, the importance of water, and health inspection compared to pastoralists. In contrast, pastoralists had better knowledge of items related to natural behavior expression, animal care, and animal suffering than mixed crop-livestock farmers. Pastoralists had 3.3-times higher odds than mixed crop-livestock farmers to have a positive attitude to train their animals without beating. KAP scores demonstrate the need for targeted training to improve animal well-being (i.e., housing, management, nutrition, disease prevention and treatment, responsible care, humane handling) across livestock holding communities in Ethiopia.

KEYWORDS

crop-livestock, item response theory, livelihoods, pastoralist, smallholder farmer

Introduction

In Ethiopia, smallholder farmers depend on livestock for food, income, and other socio-economic benefits (1). Most livestock production in this setting can be classified as low input and is largely extensive. Improved animal welfare in this context is strongly linked to farm productivity, food security, and human health (2–4). However, the welfare of livestock managed under these farming systems can be poor as a result of several factors including limited resources, inadequate knowledge and skills of animal keepers, and weak veterinary services (5–7). This subsequently limits the potential contribution of livestock sectors toward food and nutritional security and improved livelihoods, both at a household level and to the national economy (8). Moreover, the health of animals and the safety of animal products are compromised due to the burden of infectious diseases and the frequent use of antibiotics (9, 10).

Livestock owners are responsible for ensuring all aspects of animal welfare, including proper management, housing, nutrition, disease prevention and treatment, animal care, human handling, and when necessary, humane killing (11). Livestock owners in Ethiopia mostly describe animal welfare as related to the biological needs of the animals but do also recognize their animals' affective state and behavioral needs (12, 13). It is not clear their knowledge of different components of animal welfare, however, nor how well they are putting these into practice.

Overall animal welfare in Ethiopia faces numerous challenges that have not been addressed. Thus, understanding welfare knowledge, attitude, and practice (KAP) among livestock keepers is an important step toward identifying the gaps in animal care and providing a proper recommendation that will help to improve animal welfare and well-being (14, 15). It is also important to assess the association between the probability of a correct response and the characteristics of the measurement tool. Methods based on Item Response Theory (IRT) provide an important description of each item (question) in the form of item parameter estimates such as difficulty and discrimination, and KAP score (16).

Here we present a novel tool to assess KAP around animal welfare amongst smallholder farming communities in Ethiopia. Understanding how animal welfare KAP items function differently in relation to certain factors is also important to develop effective community training initiatives and policy directions. In the case of this study, we aimed to understand if the factors of farming practice, gender, and environmental differences were influencing animal welfare KAP.

Methods

Study design and setting

A cross-sectional study was conducted from February to August 2021 in four purposefully selected districts in two regional states of Ethiopia. Humbo was selected from Southern Nation Nationality and People (SNNP) regional state; Dugda, Moyale, and Miyo were selected from Oromia regional state (Figure 1). Tree coverage differs throughout the districts. In each district, two kebeles (which are the smallest administrative unit in Ethiopia) with relatively good tree access or relatively limited tree access areas were purposively targeted for data collection.

Humbo and Dugda districts represent the mixed crop-livestock production system and were selected for this study based on their potential for agroforestry farming. Humbo and Dugda districts have a total population of 125,000 (50% female) and 145,000 (49% female), respectively (17). In both districts, rural livelihood mainly depends on a mixed crop-livestock farming system in which farmers produce crops, for household consumption and sale and rear livestock simultaneously. Dugda has three agro-climatic zones: arid, semi-arid, and sub-humid. Whereas, Humbo has two agro-climatic zones: arid and semi-arid agro-climatic zones (18).

Moyale and Miyo districts of the Borana zone represent the traditional lowland pastoral livestock production systems. Livestock keeping is the predominant economic activity in the area, where the communities adopt seasonal mobility as a strategy for coping with seasonally available water and pasture resources. The total population of the Miyo and Moyale districts is 52,000 (50% female) and 31,000 (48% female), respectively (17). These areas were found in the southern arid and semi-arid parts of Ethiopia (18); a region that is highly vulnerable to climate change and recurring drought impacts resulting in widespread animal death, food insecurity, and conflicts. Moreover, population pressure, bush encroachment, and rangeland degradation are some of the added factors affecting the community. By comparison to Dugda and Humbo, they also suffer from poor access to health services and education, with few opportunities to engage in income-generating activities other than livestock (19).

Data collection tool

The data collection tool covering a range of species and welfare topics was developed to collect relevant information to measure participants' KAP on animal welfare. The KAP questionnaire consists of a set of 34 items (questions) to determine knowledge (11 items), attitudes (10 items), and practice (13 items) among the respondents. The KAP questions covered a range of species and welfare topics including health (11) and nutrition (7), environment (2), behavioral (6) and mental/emotional state (8) dimensions of animal welfare. The responses of the items were measured on a Likert scale ranging from 1 to 5 (1 = strongly disagree to 5 = strongly agree) with higher scores indicating the most desired/undesired responses (Table 1). The socio-demographic characteristics of study participants such as age, gender, and occupation were also captured. While developing the tool,

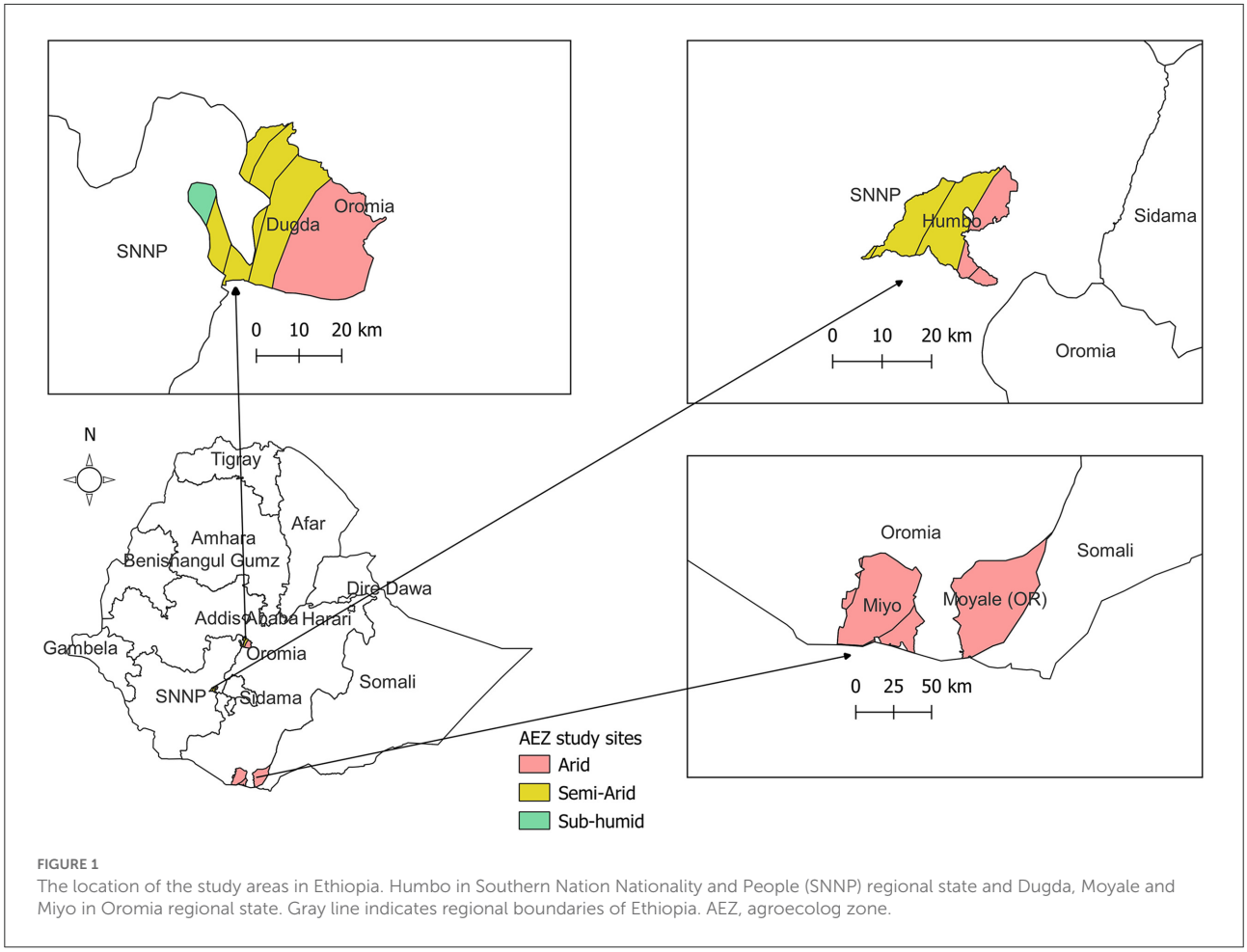

FIGURE 1
The location of the study areas in Ethiopia. Humbo in Southern Nation Nationality and People (SNNP) regional state and Dugda, Moyale and Miyo in Oromia regional state. Gray line indicates regional boundaries of Ethiopia. AEZ, agroecolog zone.

we reviewed different literature dealing with animal welfare and applied insights gained through community conversations with Ethiopian livestock owners from similar regions (12, 20). The questionnaire was then reviewed and assessed by subject experts and the research team for its content, design, validity, relevance, and understanding of the questionnaire items. Then, the questionnaire was pre-tested with farmers who were not included in the study population. The contents of the data collection tools were slightly modified based on the pilot survey, and suggestions from various people were included. The questionnaire was uploaded to a server for digital data collection using the open data kit (ODK) app installed onto tablets.

Participants and data collection process

This KAP assessment was part of a larger baseline survey that was conducted to determine the welfare condition of the humans and animals in households across sites varying in agro-climatic zones and level of tree coverage. The information was collected from a total of 197 (106 men and 91 women) smallholder farmers across all the districts. The interviews were conducted in local languages by a trained expert from the National Agricultural Research System (NARS) from the respective study sites (21).

The study participant owned different animal species including cattle, sheep, goats, poultry, and donkeys, and camels were owned in pastoral households. The mean (median) herd/flock sizes owned by farmers included in the study ranged from 12.0 (median = 9) for cattle and 1 (median =1) for equine (Table 2).

Ethical approval and consent from participants

This study received ethical approval from the International Livestock Research Institute Institutional Research Ethics Committee (ILRI IREC2020-43). The farmers/pastoralists were informed about the purpose of the study and the approximate time the interview will take, their right to withdraw at any time, and their anonymity and informed consent were obtained.

TABLE 1 Description of items used to assess the knowledge, attitude, and practice among livestock owners.

Item code	Item description	Responses
k	**Animal welfare knowledge**	1=strongly disagree, 2=disagree/disagree to some extent, 3=neutral (neither agree or disagree), 4=agree/agree to some extent, and 5=strongly agree
k1	Able to assess the amount and quality of feed	
k2	Free grazing is important for the animals	
k3	Animals need of sufficient, clean and comfortable area to lie down	
k4	Animals are sentient	
k5	Able to tell when animals are hungry or unhappy	
k6	Owner care affects how animals grow/produce	
k7	Bad handling leads to fear toward the owner	
k8	Untreated injuries affect the well-being and productivity of animals	
k9	Without enough water, animals' do not grow and produce milk	
k10	Animals can suffer from physical pain	
k11	I can quickly tell when one of my animals is sick	
at	**Animal welfare attitude**	
at1	I am confident in getting my animals to move where I want	
at2	My animals will learn more from being hit than instructed	
at3	Animals need to be able to perform their natural behaviors	
at4	I feel confident treating injuries that my animal may have	
at5	My animals must have enough water to drink	
at6	It is important to assess the health and welfare of my animals every day	
at7	I cannot influence how healthy my animals are	
at8	It is important to me that I care for my animals well	
at9	I believe my animals are happy and healthy	
at10	Animals need to feel safe in my care	
p	**Welfare practice scale**	
p1	My animals get enough to feed every day	
p2	I monitor the growth/weight of my animals	
p3	When I notice my animals are hungry, I act	
p4	My animals have a chance to move freely every day	
p5	I need to beat my animals to get them to do what I want	
p6	When I see an injury on my animal, I treat it	
p7	I consult with a trained health service provider when my animal is sick or injured	
p8	My animals can drink water whenever they want	
p9	It is common for my adult animals to get sick	
p11	My animals are exposed to heat or kept in poor housing.	
p12	Some of my animals suffer from lameness.	
p13	My animals walked long distances when selling and buying	

Data analyses

Descriptive statistics were used to summarize the data. Items were measured on a Likert scale ranging from 1 to 5 (1 strongly disagree to 5 strongly agree). This scale was then recorded for analysis into a binary outcome (0/1) in which the correct or desirable responses were assigned a score of "1" and incorrect or undesirable responses were assigned "0." Strongly agree with positive responses and strongly disagree with negative responses were categorized as desirable responses. For the attitude section, responses of "neither disagree nor agree" were excluded from the analysis, but this type of response was categorized as undesirable for knowledge and practice items. The item mean scores were transformed to a 0–100 scale for ease of interpretation. Unidimensionality of each scale, respectively knowledge, attitude, and practice, were determined

TABLE 2 Mean (median) number of animal species owned by study participants according to production systems.

Animal species	Production system					
	Mixed crop-livestock		Pastoral		Total	
	Mean	Median	Mean	Median	Mean	Median
Cattle	8.2	7	14.6	11	12.0	9
Sheep	3.3	1	8.8	5	6.6	3
Goat	4.6	3	13.8	11	10.1	6
Equine	1.3	1	0.9	0	1.1	1
Poultry	5.8	5	2.6	1	3.9	2
Camel	.	.	2.4	0	2.4	0

using factor analysis assessing the size of eigenvalues, scree plots, and the magnitude of item loading from the first factor. The internal consistency of the scale was tested using Chronbach's alpha, to assess how good a scale is at measuring a concept. Chronbach's alpha ≥ 0.7 was considered to reflect good reliability of the scale (22, 23). Items for which a single underlying latent variable could not be measured were excluded from further analysis.

In Item response theory (IRT) modeling, the probability of a correct response to an item by an individual is assessed by the values of the latent variable (theta) and the characteristics of the item (24, 25). Two-parameter logistic regression IRT (2 PL) was fitted after confirming the unidimensionality assumption of the scale. Both an item's difficulty level and discrimination ability were evaluated. Item difficulty is also called item location parameter (b), which determined the 50 probabilities of responding correctly to a specific item given the respondent's ability. An item with a low level of difficulty (i.e., an easy item) was more likely to be answered correctly than an item with a high difficulty level. Item difficulty level between -4 and $+4$ was considered acceptable.

Item discrimination parameter (a), along with a plot of all item-specific information characteristic curves, allowed the determination of how well the items discriminate farmers/pastoralists with different levels of animal welfare knowledge, attitudes toward, and practices (16). The relationship between an individual's underlying trait and the probability of answering each question correctly was visualized using item characteristic curves (ICCs). Items with a ≤ 0.7 or excessively flat ICC curves were considered low discriminatory power and excluded from further analysis.

Item and test information function curves graphically depicted the amount of information each item and scale provided against a participant latent trait. The Item information function (IIF) for the 2pl model combined two-item parameters to indicate the amount of information provided by each item along with the θ value. The test characteristic curve (TCC) graph was plotted to show the expected scores from individuals with different latent trait levels. Scatterplots were added to TCC plots to assess the fit of expected scores with observed scores (26, 27).

Group IRT analyses were conducted to determine the probabilities of answering a given scale according to respondents' farming practices, gender, and tree access. Differential item functioning (DIF) analyses were performed to determine the likelihood of individual items responding differently with two groups (28, 29).

Mantel-Haenszel Tests (MH) were used to determine whether an item exhibited uniform DIF between the observed groups (farming practice, gender, tree access). That is, whether an item was answered in a "better" way by one group relative to the other for all values of the latent trait. Data analyses were carried out using STATA software program version 16 (Texas, USA).

Results

Demographic characteristics of the study participants

The demographic characteristics of the respondents are presented in Table 3. The mean age of participants was 42.5 (SD $\pm$ 15.3) years. Among the participants, 106 (53.8%) were men and 91 (46.2%) were women. Regarding respondents' main activities, 117 (59.4%) and 80 (40.6%) of them were pastoralists and mixed crop-livestock farmers, respectively.

Livestock species and number owned

Of the total interviewed households, 100% kept cattle, 69.5% kept sheep, 82.23% kept goats, 62.94% kept poultry, and 53.81% kept equine. Except for cattle, the ownership of other species significantly ($P \leq 0.05$) varied between production systems

TABLE 3 Sociodemographic characteristics of study participants in the pastoral and mixed crop-livestock production system.

Categories	Production system, No (%)		Overall
	Pastoral	Mixed crop-livestock	
Mean Age	42.6	42.3	42.5
Male	49 (41.9)	57 (71.3)	106 (53.8)
Female	68 (58.1)	23 (28.8)	91 (46.2)
Less tree accesses	69 (59.0)	40 (50.0)	109 (55.3)
Good tree accesses	48(41.0)	40 (50.0)	88 (44.7)

Values in the brackets represent the standard deviation.

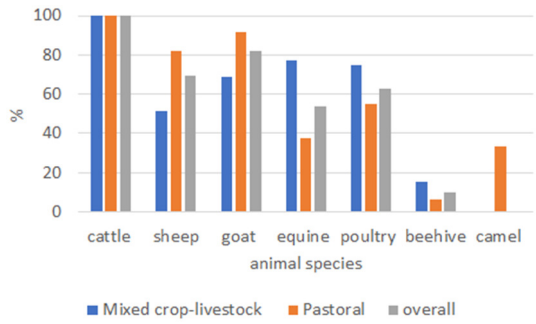

FIGURE 2
Livestock ownership status of respondents based on production system in Ethiopia.

(Figure 2). Additionally, 9.64% of the participants had beehives in their backyards. Regarding species diversity, the majority (63.5%) of the households owned more than three animal species on their farm.

Psychometric properties of items and scales

From the factor analysis, all KAP scales were sufficiently unidimensional for the application of unidimensional IRT analysis and had good internal consistency reliability with Cronbach's α (Tables 4–6). Two items from the practice scale (p9 and p13) had loading below 3 and subsequently were not used in IRT parameter estimation. The discrimination (a) and difficulty (b) parameters from the IRT analysis of the KAP scale are presented in Tables 4–6, respectively. Item discrimination parameters ranged from 0.8 to 3.7 for knowledge, 1.0 to 2.1 for attitude, and 1.1 to 2.4 for practice scale. The difficulty parameters ranged from −0.2 to 0.8 for knowledge, and from 0.3 to 2.3 for the practice scale, suggesting that knowledge questions were easy to be answered correctly by at least 50% of respondents.

Test information functions of the KAP scales are displayed in Figure 3. The TCC plot shows the observed total score values vs. ability (expected score) overlaid (Figure 4). Evidence of good fit was observed for individuals with the latent trait between −0.8 and 1.4, for the knowledge scale, and between −1 and 1.5 for the attitude scale. However, the observed total score shows evidence of deviation from the expected score, particularly for individuals with a latent practice level between −0.6 and 0.5 on the practice scale.

Animal welfare knowledge

The total knowledge score ranged from 0 (incorrect) to 11 (all correct) and the mean (±SD) score was 4.8 (±3.2). The list of all items, along with the percentage of correct answers, aggregated by the production system is shown in Figure 5. Overall, the percentage of correct responses was 43.5%. Mixed crop-livestock farmers answered more correct responses than pastoralists (54.5 vs. 36.0%). The mean percent of correct responses was similar for male (43.4%) and female (43.6%) respondents (Supplementary Table 1). The respondents recorded the lowest score for item k7 (27.91%) which related to the animal-human relationship ("bad handling leads to fear toward the owner") and the highest score (56.3%) for item k9 related to the biological needs of the animal ("without enough water, animals" do not grow and produce milk'). The desired percent of correct knowledge, i.e., an average of responses above 50%, was recorded for two statements only (item k4 "Animals are sentient" and k9) (Figure 5).

Group IRT analysis result showed that mixed crop-livestock farmers had better knowledge of animal welfare than pastoralists with a mean θ value of 1.2 (95% CI: 0.7, 1.7) and a variance of θ of 1.9 (95% CI: 1, 3.89), which had expected values 0 and 1, respectively. The difference is statistically significant ($p = 0.00$). However, there was no significant knowledge difference between male and women livestock keepers ($p = 0.91$) with a mean θ value of −0.02 (95% CI: −0.33, 0.29) and a variance of θ of 1.05 (95% CI: 0.57, 1.98).

The MH DIF test result showed that all items in the knowledge scale demonstrated significant differential item functioning, except for three items related to animal feed resource (k1), housing (k3), and wound management (k8) (Table 4). However, none of the items showed DIF related to gender and tree access. Mixed crop-livestock farmers have better knowledge of items related nutrition condition of the animal (k5), the animal-human relationship (k7), the importance of water for growth and milk production (k9), and health inspection (k11) than pastoralists. Pastoralists have better knowledge of items related to natural behavior expression

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TABLE 4 Cronbach's alpha, IRT parameter estimates and uniform DIF for the animal welfare knowledge items.

Item code	Item description	Cronbach's α	*a*	*b*	OR	95% CI		*P*-value
k1	Able to assess the amount and quality of feed	0.81	0.8	0.4	0.5	0.2	1.0	0.09
k2	Free grazing is important for the animals	0.81	0.8	0.5	0.2	0.1	0.5	0.00
k3	Animals need of sufficient, clean and comfortable area to lie down	0.80	1.3	0.2	0.6	0.3	1.3	0.26
k4	Animals are sentient	0.79	1.8	−0.2	0.4	0.1	0.9	0.04
k5	Able to tell when animals are hungry or unhappy	0.79	2.1	0.3	13.0	4.8	35.2	0.00
k6	Owner care affects how animals grow/produce	0.80	1.7	0.6	0.02	0.0	0.2	0.00
k7	Bad handling leads to fear toward the owner	0.80	1.7	0.8	3.3	1.5	7.5	0.00
k8	Untreated injuries affect the well-being and productivity of animals	0.78	3.7	0.1	0.8	0.3	2.0	0.75
k9	Without enough water, animals' do not grow and produce milk	0.78	3.4	−0.2	7.1	2.3	21.5	0.00
k10	Animals can suffer from physical pain	0.81	1.0	0.1	0.1	0.0	0.2	0.00
k11	I can quickly tell when one of my animals is sick	0.81	1.5	0.5	26.0	9.8	69.2	0.00
k	**Animal welfare knowledge scale**	**0.81**	**1.8**	**0.3**				

a, discrimination parameter; b, difficulty parameter; OR, odds ratio; CI, confidence interval. The bold values indicate the average value of the items or overall value of the knowledge, attitude and practice scales.

(k2), animal care (k6), and animal suffering (k10) than mixed crop-livestock farmers. Likelihoods of mixed crop-livestock farmers to respond correctly to items k5, k7, k9, and k11 correctly were 13, 3.3, 7.1, and 26-times higher than that of pastoralists, respectively. Nevertheless, the pastoralist had 50, 5, and 10-times higher odds to respond to items k2, k6, and k10 correctly, respectively.

Animal welfare attitude

From the total of 10 points, the mean (±SD) score of desirable attitudes was 3.4 (±0.2). The list of all questions, along with the percent of desired responses aggregated by the livestock production system, is shown in Figure 6. Overall, the percentage of correct responses for the attitude scale was 35.7%. Mixed-crop livestock farmers answered more questions "correctly" than pastoralists (43.7 vs. 30.1%). A slightly higher mean percent of desired responses were obtained for female (37.3%) than male (34.3%) respondents (Supplementary Table 1). The percent of desired responses for the individual question ranged from 23.4% for item at9 ("my animals are happy and healthy") to 52.6% for item at5 ("my animals must have enough water to drink"). The respondents scored above 50% desired response for only one statement (item at5).

The group IRT analysis result showed that mixed crop-livestock farmers had a better attitude toward animal welfare than pastoralists ($p = 0.002$) with a mean θ value of 0.54 (95% CI: 0.2–0.9) and variance of θ of 1.1 (95% CI: 0.5–2.1). Nevertheless, attitudes toward animal welfare did not show significant differences between men and women and respondents with good and less tree access.

From the MH DIF test result, only one item from the attitude scale, at2 ("my animals will learn more from being hit than instructed") had significant differential item functioning related to the production system (Table 5), and none of the items had DIF related to tree access and gender. Pastoralists had 3.3-times higher odds than mixed crop-livestock farmers to have a positive attitude about how to train their animals (OR = 0.3, 95% CI = 0.1–0.9, $p = 0.05$).

Animal welfare practices

From a total of 13 points, the mean (±SD) score of correct practice was 3.2 (± 0.2). The list of all practice questions, along with the percent of correct responses aggregated by the livestock production system, is shown in Figure 7. Overall, the mean percent of correct responses for self-reported practice was 26.4%. A slightly higher mean percentage of correct responses was

TABLE 5 Cronbach's alpha, IRT parameter estimates, and uniform DIF for animal welfare attitude items.

Item code	Items descriptions	Cronbach's α	*a*	*b*	OR	95%CI		*P*-value
at1	I am confident in getting my animals to move where I want	0.76	1.62	0.9	1.3	0.5	3.3	0.80
at2*	My animals will learn more from being hit than instructed	0.78	0.86	1.5	0.3	0.1	0.9	0.05
at3	Animals need to be able to perform their natural behaviors	0.75	1.89	0.3	0.6	0.2	1.6	0.40
at4	I feel confident treating injuries that my animal may have	0.77	1.52	0.9	2.6	0.9	7.7	0.13
at5	My animals must have enough water to drink	0.75	2.41	0.0	1.0	0.4	2.6	0.85
at6	It is important to assess the health and welfare of my animals every day	0.75	2.37	0.0	1.6	0.7	4.0	0.41
at7*	I cannot influence how healthy my animals are	0.77	0.99	0.9	0.9	0.4	2.2	0.99
at8	It is important to me that I care for my animals well	0.75	1.84	0.2	2.2	0.8	5.9	0.19
at9	I believe my animals are happy and healthy	0.78	0.91	1.5	0.7	0.2	2.0	0.64
at10	Animals need to feel safe in my care	0.76	1.66	0.5	0.7	0.2	2.0	0.67
at	**Animal welfare attitude**	**0.78**	**1.61**	**0.7**				

Scale reversed; a, discrimination parameter; b, difficulty parameter; OR, odds ratio; CI, confidence interval. The bold values indicate the average value of the items or overall value of the knowledge, attitude and practice scales.

obtained for male (26.8%) than for female (22.1%) respondents (Supplementary Table 1). The mean correct response ranged from 10.2% for item p1 ("my animals get enough to feed every day") to 43.7% for item p3 ("when I notice my animals are hungry, I act") for individual items. The respondents scored all the statements below the required average (50%) animal practice level.

The group IRT analysis result showed mixed crop-livestock farmers had better self-reported animal welfare practices than pastoralists ($p = 0.00$), with a mean θ value of 1.2 (95% CI: 0.8–1.5) and variance of θ of 0.7 (95% CI: 0.3–1.5).

The result of the MH DIF test for practice scale items showed that only one item, p8 ("my animals can drink water whenever they want") had significant differential item functioning related to the production system (Table 6) and none of the items had DIF related to tree access and gender. Mixed crop-livestock farmers had a 3.4 times higher probability to provide water for their animals whenever they want than pastoralists (OR = 3.4, 95% CI = 1.3–8.9, $p = 0.01$).

Correlation between respondents' knowledge, attitude, and practice

A Pearson correlation analysis was conducted to assess the relationship between the total score of the KAP scales. Figure 8 shows the relationship between knowledge, attitude, and practice. There was a significant positive association between respondents' knowledge and attitude toward animal welfare ($r = 0.74$, $p = 0.00$), suggesting having appropriate knowledge explains 54.8% of the positive attitude the respondents developed. Similarly, there was a strong positive association between respondents' knowledge and self-reported practice ($r = 0.57$, $p = 0.00$), suggesting having appropriate knowledge explains 32.5% of good animal welfare practices. Good practices also had a strong and positive correlation with desirable attitudes ($r = 0.57$, $p = 0.00$), having a desirable attitude explaining 32.5% of good animal welfare practices.

Discussion

This study provided a summary of animal welfare KAP results and evaluate the reliability of the assessment tools in three communities in Ethiopia. The finding showed that a higher score was recorded for the animal welfare knowledge scale followed by attitude. However, overall, the livestock owners had inadequate knowledge of animal welfare, undesirable attitude toward the animals they handle, and suboptimal animal welfare practices.

Animal welfare KAP from across Africa are limited. Another study has documented a lack of deep knowledge of most of the critical animal welfare issues, undesirable attitudes, and poor

TABLE 6 Cronbach's alpha, IRT parameter estimates, and uniform DIF for animal welfare practice items.

Item code	Item description	Cronbach's α	*a*	*b*	OR	95% CI		*P*-value
p1	My animals get enough to feed every day	0.8	1.2	2.3	2.4	0.6	8.6	0.28
p2	I monitor the growth/weight of my animals	0.8	1.6	0.9	1.2	0.5	2.7	0.87
p3	When I notice my animals are hungry, I act	0.8	1.5	0.3	0.8	0.4	1.9	0.80
p4	My animals have a chance to move freely every day	0.8	1.5	1.0	0.6	0.2	1.5	0.40
p5*	I need to beat my animals to get them to do what I want	0.8	2.1	1.1	0.5	0.2	1.6	0.34
p6	When I see an injury on my animal, I treat it	0.8	1.1	0.4	0.7	0.3	1.4	0.39
p7	I consult with a trained health service provider when my animal is sick or injured	0.8	1.6	0.7	1.4	0.6	3.3	0.62
p8	My animals can drink water whenever they want	0.7	2.3	0.8	3.4	1.3	8.9	0.01
p9*	It is common for my adult animals to get sick	0.7	0.9	2.7	0.1	0.0	0.6	0.00
p10*	When an animal is sick, I cannot influence its recovery	0.7	1.9	0.8	1.2	0.5	2.9	0.81
p11*	My animals are exposed to heat or kept in poor housing.	0.8	1.7	1.5	0.3	0.1	1.1	0.10
p12*	Some of my animals suffer from lameness.	0.8	1.1	1.1	1.1	0.5	2.5	0.95
p13*	My animals walked long distances when selling and buying	0.7	1.1	2.1	0.3	0.1	1.0	0.07
p	**Welfare practice scale**	**0.8**	**1.5**	**1.2**				

*Scale reversed; a, discrimination parameter; b, difficulty parameter; OR, odds ratio; CI, confidence interval. The bold values indicate the average value of the items or overall value of the knowledge, attitude and practice scales.

welfare practices among stock persons in Kenya (15). The roles of animal owners to abattoir stock people are markedly different, in terms of responsibility, ownership, and connection to animals. The poor attitude and practices toward animal welfare recorded in this study might be related to inadequate knowledge, which might relate to low awareness of the farming community on the physical, biological, and behavioral requirements of the animals. A lack of appropriate information on animal welfare may prevent owners from developing a positive attitude toward animal welfare (30–32) and as a result, fail to improve practice (33, 34). Access to animal welfare-related information and training initiatives to improve livestock welfare is considered important to increase the awareness of the farmers on animal welfare (4, 35), and seems to be lacking in the Ethiopian agricultural extension system (36).

The result of this study showed that mixed crop-livestock farmers had a better KAP score than pastoralists. Mixed crop-livestock farmers have better access to extension and veterinary services which enable them to have a better awareness of animal care and management and implement animal health-related activities than pastoralists (37). This may in part explain the geographical differences. Pastoralists are mobile with their livestock and move in response (at least in part) to the availability of feed and water resources. This movement process can hamper pastoralist access to information and basic animal health care and extension services (38, 39). Public-private partnership (PPP) model which creates enabling environments for efficient use of available resources or to expand coverage of veterinary health services (40, 41) is one approach that can be promoted in pastoral areas to address challenges in animal health and could include animal welfare perspectives as part of the PPP contract.

The difference in the perceptions of the farmers on animal welfare is influenced by geographical, economic, social and environmental and cultural, and religious beliefs, and may often be different from the welfare needs of the animal (6,

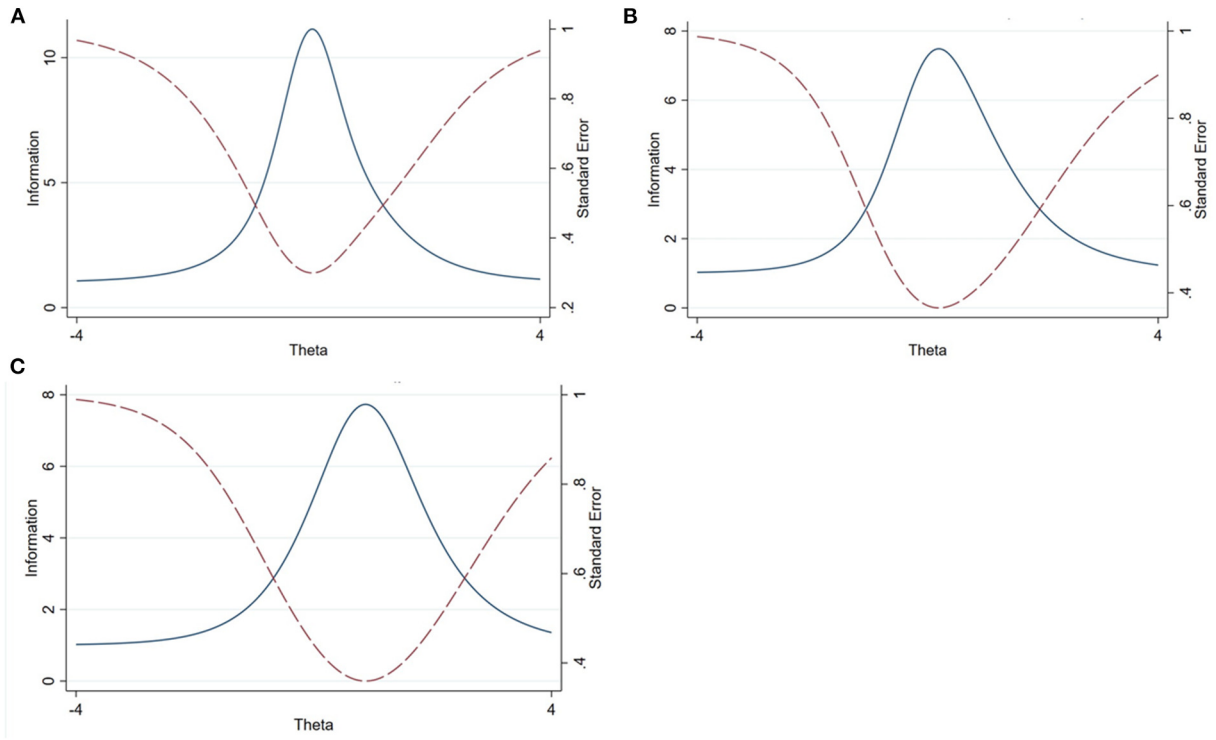

FIGURE 3
Test information function for animal welfare knowledge **(A)**, attitude **(B)**, and practice **(C)** scale. Blue line indicates "Test information" and red dot line indicate "Standard error." The questions provided maximum information for respondents with knowledge level between −1 to 1.5, attitude level between −1 to 2 and practice level of −0.5 to 2.6.

14, 42). This may further, in part, explain some differences in crop-livestock and pastoralist KAP score. Community members attach different values or meanings to animals depending on the purpose of the animals and their relationships with the animals. For instance, women value and have a closer relationship with dairy cows, while men focus on social status and prestige, and thus attach more value to cattle and their number (12). Mixed crop farmers have frequent interaction with their animals due to the smaller herd size and the use of animals for crop agriculture and transport (5). The pastoralists have intimate knowledge and connection with their animals, and the animals in the pastoral production system tend to move freely within the rangeland in the search of feed and water and exhibit their natural behaviors without restriction (43, 44). These different roles that animals play in the two different agricultural systems relate to the difference in responses seen in the current paper.

The prevalence of poor practices recorded in this study related to animal feed needs enormous improvement. Under an Ethiopian extensive production system, the livestock often spends the whole day without enough feed and water (45, 46). Moreover, pastoral production systems are practiced in drylands agroecosystems where multiple stressors such as excessive heat, and the need to walk long distances to source feed and water create further welfare compromises for the animal (47). Feed and water resource improvement strategies, such as silvopastoral or agropastoral farming systems, have been demonstrated to have a positive impact on animal welfare (48, 49). These systems can be promoted and adopted across both pastoralist-dominated and crop-livestock landscapes. Agro-ecologically appropriate tree presence in both crop-livestock and pastoral systems is likely to have an encouraging influence on animal welfare and productivity, particularly by allowing the expression of natural behavior, and providing shade and quality feeds (49, 50).

Inappropriate management practices, such as beating, were both highlighted in this study and have been previously described in similar settings, especially in the mixed crop-livestock production system (5, 42). Actions to improve the empathy of owners toward their animals, and encourage low-stress handling practices, could further help to improve animal welfare.

All knowledge, attitude, and practice set of questions used in this study met the unidimensional assumption of the IRT model. It showed good reliability with acceptable Cronbach's alpha value and fit well with the scale. From the parameters estimate, the knowledge scale had a higher discrimination ability than the attitude and practice scales. The statements in the knowledge

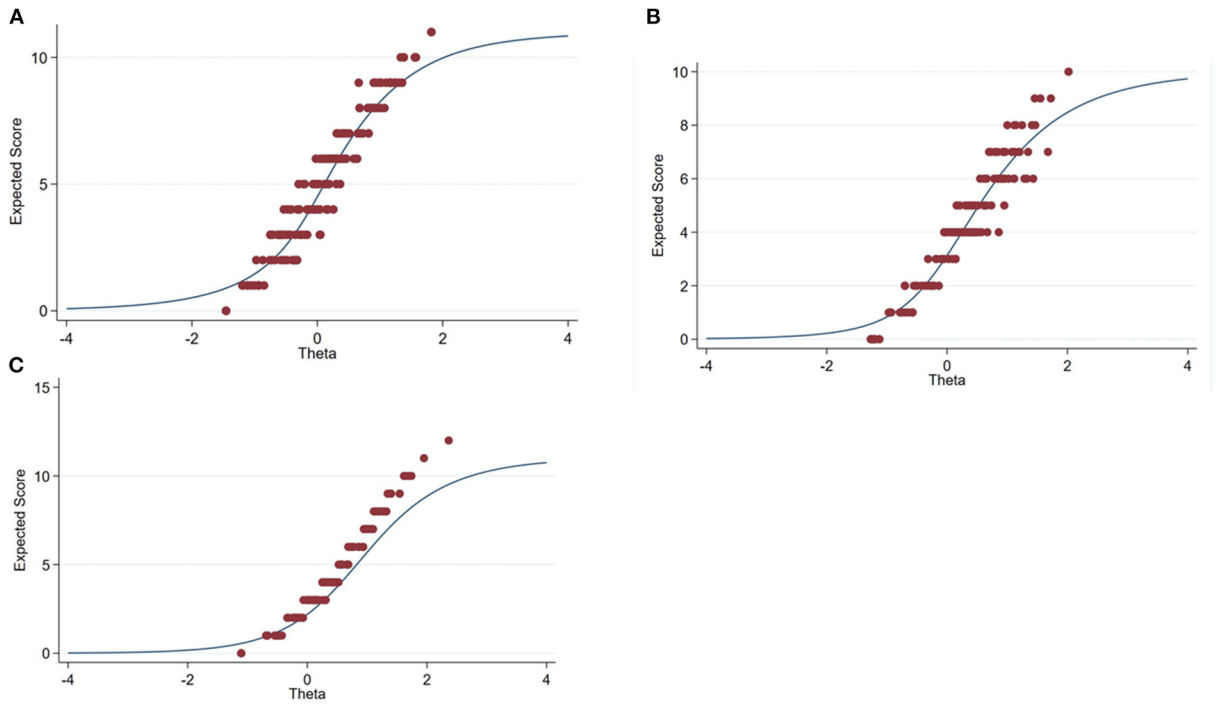

FIGURE 4
Test characteristic curve for animal welfare knowledge **(A)**, attitude **(B)**, and practice **(C)** with an added plot (red spots) of the summated score vs. ability (predicted score). Blue line indicates "expected score" and red dot indicate "total score." Observed total score and expected score showed good fit for individual with knowledge between −0.8 and 1.4 and attitude level between −1 and 1.5 for the scale.

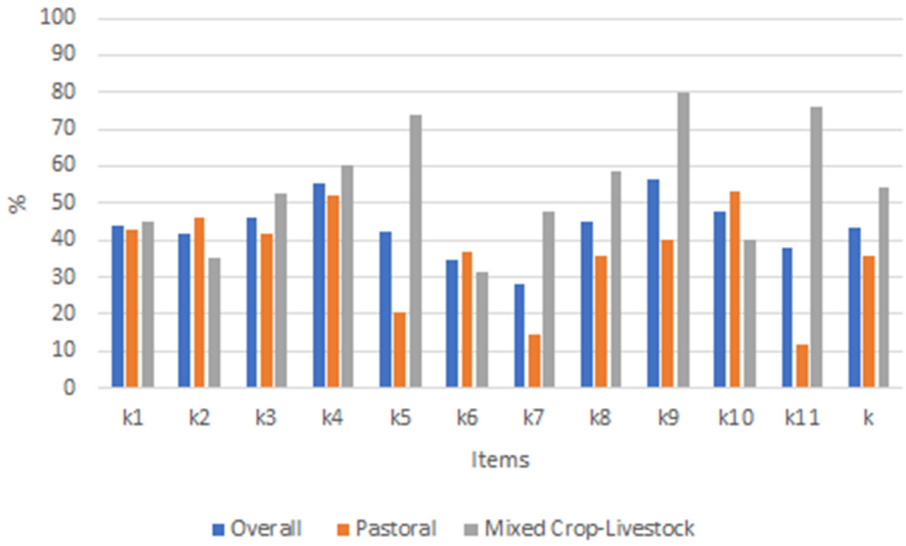

FIGURE 5
Percent of correct responses for animal welfare knowledge items aggregated by production system in Ethiopia.

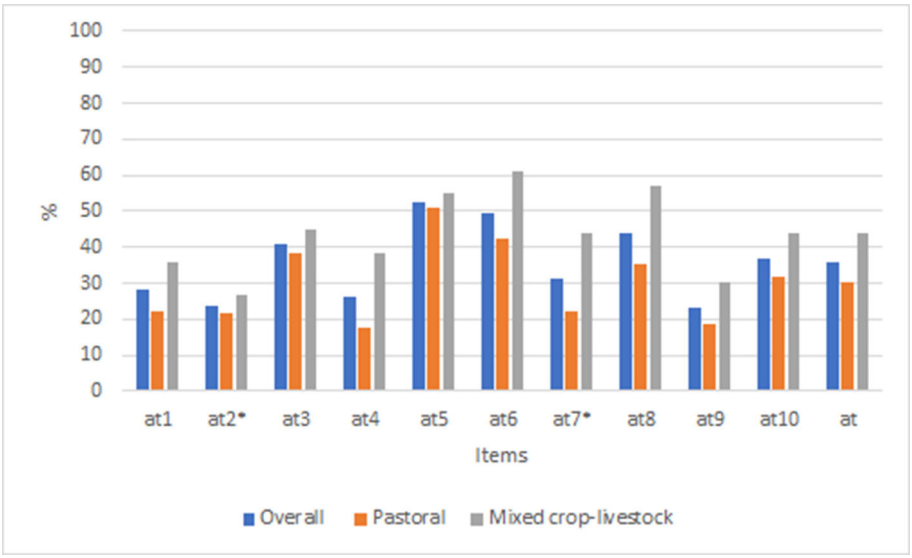

FIGURE 6
Percent of correct responses for animal welfare attitude items aggregated by production system in Ethiopia. Items with "*" indicate the scale were reversed.

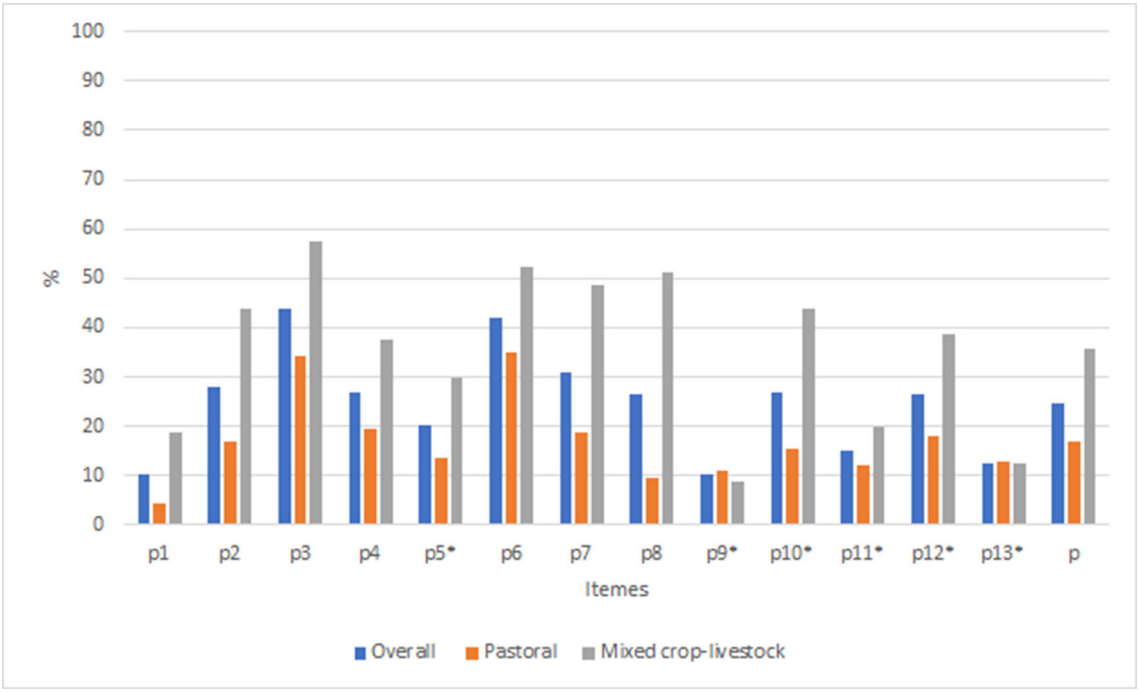

FIGURE 7
Percent of correct responses for animal welfare practice items aggregated by production system in Ethiopia. Items with "*" indicate the scale were reversed.

scale were relatively easy for the respondents with higher probabilities of responding to them correctly. This implies the livestock owners had better animal welfare knowledge than a positive attitude and good practice which might be acquired through experience, training, from opinion leaders, and peer-to-peer learning. It also likely reflects the barriers that owners face to address these known animal welfare needs. For example, an animal owner can know the correct answer to K9 "Without

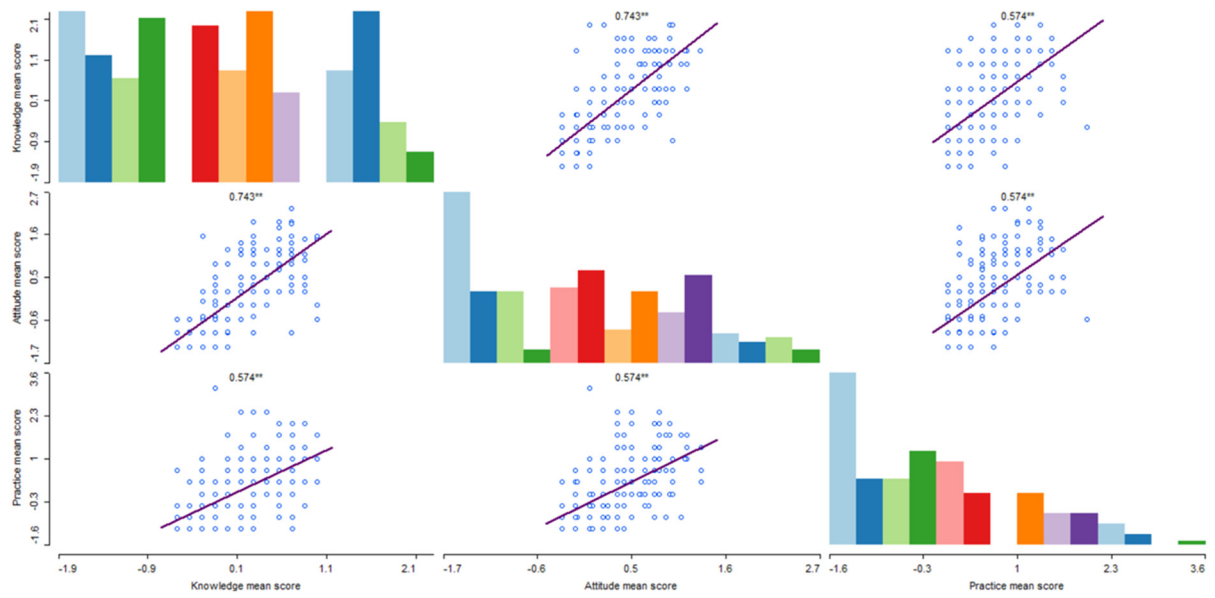

FIGURE 8
Correlation matrix which show the relationship of KAP score among smallholder farmers in Ethiopia.

enough water, animals" do not grow and produce milk', but be unable to turn it into practice (i.e., P8 "My animals can drink water whenever they want").

This is important to consider when turning the results from the current study into practice change to improve animal welfare. Community-based engagement and learning processes, called Community Conversations have the potential to both increase awareness of an issue and generate community-led steps to address issues (12). Community Conversations collectively identify community strengths, knowledge gaps, and constraints, analyze community values and practices, and explore strategies for addressing challenges (20). This approach can make Community Conversations a particularly useful tool for animal welfare improvements because it creates awareness of issues and enables the participants to then create solutions. For example, participants can become more aware of issues with poor animal handling and limited water availability, and then pledge to take a gentler approach to handle and build community troughs to improve water access for animals while grazing.

This study was not without limitations. The study participants were selected purposively based on their tree access which makes the results difficult to generalize to other small holders farmers across the vast agroecology and production systems of Ethiopia. Future studies with randomly selected participants across different agroecology and production system of Ethiopia should be conducted. A single-visit self-report interview approach may lead to a concept called social desirability bias (51) where some of the study participants describe actions that do not always reflect their actual practices. Further studies which longitudinally measure livestock owners' routine animal management practices and their impact on the welfare indicators of their animals should be considered.

Conclusion

This study found a positive correlation between the knowledge of the farmers and their attitude toward animal welfare and self-reported practices. This implied positive attitude and good animal welfare practice can be achieved through appropriate training which improves the awareness of the farmers on the biological, physical, and mental needs of their animals. The livestock production system influenced livestock keeper's animal welfare KAP, and it is likely that this is related to resource availability, and potentially due to different approaches in livestock ownership. The developed questionnaire had satisfactory psychometric properties in terms of measuring animal welfare KAP in Ethiopian smallholder farmers, making it suitable for the measurement of the impact of the intervention on animal welfare. It is also recognized that the ability to intervene to improve animal welfare may be limited, depending on the owners' production system and resources.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

RD, BW, TB, and MM conceived the study. GA and TB implemented the study. GA, EG, TB, JJ, and JM followed up and monitored data collection. TK-J supervise the overall implementation of the project activities. GA analyzed the data and prepared the first draft of the manuscript with RD. All authors made contributions to the conception, design, and revision of the manuscript. All authors reviewed and approved the final version.

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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.2022.1006505/full#supplementary-material

References

1. Desta H, Alemu B, Kinati W, Abenakyo A, Eerdewijk A Van, Wieland B. Contribution of small ruminants to food security for Ethiopian smallholder farmers. *Small Rumin Res.* (2020) 184:106064. doi: 10.1016/j.smallrumres.2020.106064

2. Doyle RE, Wieland B, Saville K, Grace D, Campbell AJD. The importance of animal welfare and veterinary services in a changing world. *Rev Sci Tech.* (2021) 40:469–81. doi: 10.20506/rst.40.2.3238

3. de Boer J, Aiking H. Considering how farm animal welfare concerns may contribute to more sustainable diets. *Appetite.* (2021) 168:105786. doi: 10.1016/j.appet.2021.105786

4. Hötzel MJ. Improving farm animal welfare: is evolution or revolution needed in production systems? *Dilemmas Anim Welf.* (2014) 2014:67–84. doi: 10.1079/9781780642161.0067

5. Rahman SA, Reed K. The management and welfare of working animals: identifying problems, seeking solutions and anticipating the future. *OIE Rev Sci Tech.* (2014) 33:197–202. doi: 10.20506/rst.33.1.2272

6. Qekwana DN, Mccrindle CME, Cendi-Goga B, Grace D. Animal welfare in Africa : strength of cultural traditions, challenges and perspectives cultural traditions and laws. In: Hild S, Schweitzer L, editors. *Animal Welfare: from Science to Law.* Paris: La Fondation Droit Animal, Éthique et Sciences (LFDA) (2019). p. 103–7.

7. Asmare B. Farm animal welfare and handling in the tropics: the ethiopia case. *Adv Agric.* (2014) 2014:1–7. doi: 10.1155/2014/428129

8. Sinclair M, Fryer C, Phillips CJC. The benefits of improving animalwelfare from the perspective of livestock stakeholders across asia. *Animals.* (2019) 9:123. doi: 10.3390/ani9040123

9. Buller H, Blokhuis H, Jensen P, Keeling L. Towards farm animal welfare and sustainability. *Animals.* (2018) 8:1–13. doi: 10.3390/ani8060081

10. Thumbi SM, Njenga MK, Marsh TL, Noh S, Otiang E, Munyua P, et al. Linking human health and livestock health: a "one-health" platform for integrated analysis of human health, livestock health, and economic welfare in livestock dependent communities. *PLoS ONE.* (2015) 10:1–18. doi: 10.1371/journal.pone.0120761

11. Madzingira O. Animal welfare considerations in food-producing animals. In: Abubakar M, Manzoor S, editors. *Animal Welfare.* London: InTech (2018).

12. Lemma M, Doyle R, Alemayehu G, Mekonnen M, Kumbe A, Wieland B. Using community conversations to explore animal welfare perceptions and practices of rural households in Ethiopia. *Front Vet Sci.* (2022) 26:9. doi: 10.3389/fvets.2022.980192

13. Ulmann SL, Bourgeois-luethi N, Mandel R. *Sheep welfare and farmers' attitudes towards animal welfare in Master' s Thesis in Agricultural Science.* (2019). p. 1–52.

14. Rollin BE. Cultural variation, animal welfare and telos. *Anim Welf.* (2007) 16(SUPPL.):129–33.

15. Wambui J, Lamuka P, Karuri E, Matofari J. Animal welfare knowledge, attitudes, and practices of stockpersons in Kenya. *Anthrozoos.* (2018) 31:397–410. doi: 10.1080/08927936.2018.1482111

16. Khosravi A, Hassan M, Hashemi H, Fotouhi A. Sleep medicine : X components of pittsburgh sleep quality index in Iranian adult population : an item response theory model. *Sleep Med X.* (2021) 3:100038. doi: 10.1016/j.sleepx.2021.100038

17. Central Statistical Agency. *Summary and Statistical Report of the 2007*. Addis Ababa, Ethiopia (2008).

18. Hurni H. *Agroecological Belts of Ethiopia. Explanatory notes on three maps at a scale of 1* (1998).

19. Gebremedhin B, Woldehanna M, Flintan F, Barbara W, Poole J. *Baseline survey report for the Regional Pastoral Livelihoods Resilience Project in Ethiopia*. (2017). p. 1–103.

20. Lemma M, Mekonnen M, Kumbe A, Demeke Y, Wieland B, Doyle R. *Community Conversations on Animal Welfare*. ILRI: Narobi, Kenya (2019).

21. Berhe T, Mekonnen M, Doyle R, Wieland B, Mokria M, Njamba H. *Synergies of Animal Welfare and Agroforestry (SAWA) field research manual*. Narobi, Kenya: International Livestock Research Institute (2022).

22. Casey ATH, Bland JM, Crockard HA. Development of a functional scoring system for rheumatoid arthritis patients with cervical myelopathy. *Ann Rheum Dis.* (1996) 55:901–6. doi: 10.1136/ard.55.12.901

23. Tavakol M, Dennick R. Making sense of cronbach's alpha. *Int J Med Educ.* (2011) 2:53–5. doi: 10.5116/ijme.4dfb.8dfd

24. Edelen MO, Reeve BB. Applying item response theory (IRT) modeling to questionnaire development, evaluation, and refinement. *Qual Life Res.* (2007). 16:5–18. doi: 10.1007/s11136-007-9198-0

25. Thissen D. Psychometrics: item response theory. In: *International Encyclopedia of the Social & Behavioral Sciences: Second Edition*. Elsevier (2015). p. 436–9.

26. Baker FB, Kim S-H. The test characteristic curve. In: Baker F, Kim S-H, editor. *The Basics ofItem Response Theory Using R, Statistics for Social and Behavioral Sciences*. Springer International Publishing (2017). p. 55–67. Available online at: http://link.springer.com/10.1007/978-3-319-54205-8_4 (accessed December 15, 2021).

27. Nguyen TH, Han HR, Kim MT, Chan KS. An introduction to item response theory for patient-reported outcome measurement. *Patient*. (2014) 7:23–35. doi: 10.1007/s40271-013-0041-0

28. Petersen MA, Groenvold M, Bjorner JB, Aaronson N, Conroy T. Use of differential item functioning analysis to assess the equivalence of translations of a questionnaire. *Qual Life Res*. (2003) 12:373–85. doi: 10.1023/a:1023488915557

29. Teresi JA, Ramirez M, Jones RN, Choi S, Crane PK. Modifying measures based on differential item functioning (DIF) impact analyses. *J Aging Health*. (2012) 24:1044–76. doi: 10.1177/0898264312436877

30. Glanville C, Abraham C, Coleman G. Human behaviour change interventions in animal care and interactive settings: a review and framework for design and evaluation. *Animals*. (2020) 10:1–40. doi: 10.3390/ani10122333

31. Pol F, Kling-Eveillard F, Champigneulle F, Fresnay E, Ducrocq M, Courboulay V. Human–animal relationship influences husbandry practices, animal welfare and productivity in pig farming. *Animal*. (2021) 15:100103. doi: 10.1016/j.animal.2020.100103

32. Kiliç I, Bozkurt Z. The relationship between farmers' perceptions and animal welfare standards in sheep farms. *Asian-Australasian J Anim Sci*. (2013) 26:1329–38. doi: 10.5713/ajas.2013.13124

33. Fraser D. Understanding animal welfare. *Acta Veterinaria Scandinavica*. (2008) 150:1–7. doi: 10.1186/1751-0147-50-S1-S1

34. Ventura G, Lorenzi V, Mazza F, Clemente GA, Iacomino C, Bertocchi L, et al. Best farming practices for the welfare of dairy cows, heifers and calves. *Animals*. (2021) 11:2645. doi: 10.3390/ani11092645

35. Paranhos da Costa MJR, Huertas SM, Gallo C, Dalla Costa OA. Strategies to promote farm animal welfare in Latin America and their effects on carcass and meat quality traits. *Meat Sci*. (2012) 92:221–6. doi: 10.1016/j.meatsci.2012.03.005

36. Berhane G, Ragasa C, Abate GT, Assefa TW. The state of agricultural extension services in Ethiopia and their contribution to agricultural productivity. *Ethiopia Strategy Support Program Working Paper*. Addis Ababa, Ethiopia (2018).

37. Gizaw S, Woldehanna M, Anteneh H, Ayledo G, Awol F, Gebreyohannes G, et al. Animal health service delivery in crop-livestock and pastoral systems in Ethiopia. *Front Vet Sci*. (2021) 8:1–14. doi: 10.3389/fvets.2021.601878

38. Hooper P. Review of animal health service delivery in the mixed crop-livestock system in Ethiopia. *LIVES Working Paper 18*. Nairobi, Kenya (2016).

39. Schelling E, Wyss K, Bechir M, Moto DD, Zinsstag J. Synergy between public health and veterinary services to deliver human and animal health interventions in rural low income settings. *BMJ*. (2005) 331:1264–7. doi: 10.1136/bmj.331.7527.1264

40. Ahmadi BV. Public private partnerships (PPPs) for efficient sustainable animal health systems and veterinary services. *Report on a Technical Item Presented to the 15th Conference of the OIE Regional Commission for the Middle East*. Abu Dhabi, United Arab Emirates (2019). doi: 10.20506/TT.2776

41. N'Guessan NB, Poupaud M, Dieuzy-Labaye I, Asfaw YT, Wieland B, Tesfu F, et al. Evaluation of public–private partnership in the veterinary domain using impact pathway methodology: in-depth case study in the poultry sector in Ethiopia. *Front Vet Sci*. (2022) 9:1–19. doi: 10.3389/fvets.2022.735269

42. Mugenda L, Croney C. Factors affecting perceptions of animal welfare in developing countries. *Animal Sciences, ag. purdue. edu/ansc. Purdue University Cooperative Extension Service*. (2019). p. 1–5.

43. Krätli S, Schareika N. Living off uncertainty: the intelligent animal production of dryland pastoralists. *Eur J Dev Res*. (2010) 22:605–22. doi: 10.1057/ejdr.2010.41

44. Krätli S, Huelsebusch C, Brooks S, Kaufmann B. Pastoralism: a critical asset for food security under global climate change. *Anim Front*. (2013) 3:42–50. doi: 10.2527/af.2013-0007

45. Temple D, Manteca X. Animal welfare in extensive production systems is still an area of concern. *Front Sustain Food Syst*. (2020) 4:545902. doi: 10.3389/fsufs.2020.545902

46. Bach A, von Keyserlingk MAG, Widowski TM, Haley D. Assessing farm animal welfare from a nutritional perspective. In: Phillips CJC, editor. *Nutrition and the Welfare of Farm Animals, Animal Welfare*. Switzerland: Springer Nature (2016). p. 115–34. doi: 10.1007/978-3-319-27356-3_6

47. Makkar HPS. *Nutrition and the Welfare of Farm Animals*. In: Phillips CJC, editor. Cham: Springer International Publishing (2016). Available online at: http://www.springer.com/series/5675 (accessed February 22, 2022).

48. Mancera KF, Zarza H, de Buen LL, García AAC, Palacios FM, Galindo F. Integrating links between tree coverage and cattle welfare in silvopastoral systems evaluation. *Agron Sustain Dev*. (2018) 38:1–9. doi: 10.1007/s13593-018-0497-3

49. Jose S, Dollinger J. Silvopasture: a sustainable livestock production system. *Agrofor Syst*. (2019) 93:1–9. doi: 10.1007/s10457-019-00366-8

50. Shrestha RK, Alavalapati JRR, Kalmbacher RS. Exploring the potential for silvopasture adoption in south-central Florida: an application of SWOT-AHP method. *Agric Syst*. (2004) 81:185–99. doi: 10.1016/j.agsy.2003.09.004

51. Grimm P. Social desirability bias. *Wiley Int Encycl Mark*. (2010) (1999):2010. doi: 10.1002/9781444316568.wiem02057

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Welfare assessment of horses and mules used in recreational and muleteer work in the Colombian coffee region

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The welfare of working equids in developing countries is sometimes threatened due to the limited resources and/or knowledge of their owners. The objective of this study is to evaluate the welfare of creole horses and mules using a validated protocol that assesses animal-based indicators. A total of 160 horses and 40 mules from three municipalities in the Colombian coffee-growing region were evaluated by means of direct observation of health and behavioral parameters. A descriptive analysis of the variables expressed in proportions was performed. Interactions between the different measurements were examined using the Chi-squared test. Spearman correlations were used to relate the measurements. Horses and mules demonstrated friendly behavior in front of the evaluators (78.13 and 61.54%, respectively); apathetic or severely depressed behavior was low (10.7 and 17.5%, $P > 0.05$). Significant differences in body condition score (BCS) were observed between mules and horses ($P < 0.05$); eighty percent of the mules and 54.4% of the horses exhibited a healthy body condition score (3 or more on a scale of 1 to 5). Less than 15% of the animals had eye problems, limb deformities, and gait abnormalities. Injuries to the head, withers, spine, ribs/flank, hindquarters, and hind legs were observed in a frequency between 12.5 and 30.43% of the animals, with a higher frequency in horses ($P < 0.05$). Weak correlations (R2 coefficient<0.5), although statistically significant, were observed between low body conditions and the presence of skin and deeper tissue lesions, systemic health abnormalities, and limb problems ($P < 0.05$). The results indicate that owners care for their animals. However, the presence of skin and deep tissue lesions, especially in horses, suggests that they are subjected to high workloads. Therefore, it is essential to train owners in aspects related to the importance of providing their equids with adequate rest periods to recover from work and develop actions to strengthen human-equine interaction.

KEYWORDS

animal well-being, working equine, developing countries, behavior, health indicators

Introduction

In many developing countries around the world, working equids contribute to family livelihoods and perform a wide variety of economic, social, and labor-reducing functions (1, 2), especially in mountainous areas, where motorized vehicles have limited access (3). However, animal welfare can be compromised due to the limited resources and/or knowledge of their owners (4). Several factors affecting the welfare of working equids have been described such as the provision of shelter, adequate feed, appropriate harnesses, veterinary care, provision of medications, and the presence and promotion of programs aimed at improving social awareness of the best animal management practices, among other aspects (2, 5).

Working horses and mules are managed differently from most stabled equids (e.g., leisure horses and horses engaged in competitive events), as they are not kept in stables equipped with special infrastructure. On the contrary, they can sometimes work long hours, pull or transport heavy loads and are often exposed to adverse environmental conditions (4). Therefore, the use of animal-based indicators to assess the welfare state of working equids is recommended because they are considered more reliable and relevant compared to resource-based indicators (6).

In 2011, the United Nations Educational, Scientific, and Cultural Organization (UNESCO) designated the Colombian Coffee Cultural Landscape as a World Heritage Site. The importance of coffee growing in the Colombian Coffee Cultural Landscape has transcended the economic aspect (7). Around this activity, a series of traditions or cultural and social manifestations have developed in the region that that has been transmitted from generation to generation. Among these traditions is the *arrieria*, an activity in which the mule driver, along with his mules and horses, participates. The mules are a species that represent "the strength and endurance of a pack animal capable of traversing the mountainous landscapes" (2). The farmer and coffee producer associations have signed veterinary assistance agreements with public universities in order to monitor the health of these animals. In this context, the objective of this study was to evaluate the welfare of mules and working horses through behavioral and health indicators in the Colombian coffee region, and identify the variables that contribute most to its variation and those that require improvement.

Materials and methods

Ethical note

All procedures related to the use and care of the animals strictly followed the Colombian regulation norm, Resolution 001634–2010 as stated by the Colombian Agricultural Institute ICA (8). Permission to conduct the study was approved by the Ethics Committee for Animal Experimentation (Act 24/06/2018, Activities with minimal risk) and the Human Ethics Committee (Act 15/06/2018) at the University of Caldas. Farmers were fully informed about the purpose of the study, and they read/listened and signed an informed consent form and authorization to allow us to use the data collected.

Characteristics of owners

Information was also obtained on the gender and level of education of the owners.

Animals and observers

The University of Caldas has had a cooperative partnership for 54 years with the department's coffee growers' cooperatives to carry out biannual medical workshops on a 5-year rotation basis (9 semesters). Previously these workshops were coordinated with the cooperatives' extension promoters, who are responsible for convening the community leaders, who in turn are responsible for disseminating the activities to the coffee growers, who participate voluntarily. In these sessions, veterinary medical consultation, reproductive diagnosis of large species, endoparasite control, administration of multivitamin supplements, small surgeries, promotion and training activities in preventive medicine, husbandry practices, sanitary programs (vaccination, feeding, good livestock practices, parasite control), among others, are carried out. Within the sanitary activities carried out by the veterinary medicine and animal science program of the University of Caldas from March to September 2019, 40 mules and 160 Creole working horses from three municipalities in the department of Caldas were studied: The municipalities being: Riosucio (n = 37, 18.4%) (Altitude: 1,729 m, Latitude: 5. 417, Longitude: −75.7 5° 25′ 1″ North, 75° 42′ 0″ West), Manzanares (n = 85, 42.3%) (Altitude: 1,933 m, Latitude: 5.25 Longitude: −75. 15 Latitude: 5° 15' 0"' North Longitude: 75° 9' 0"' West) and Pennsylvania (n = 79, 39.3%9 (Altitude: 2,165 m, Latitude: 5.383, Longitude: −75.1675° 22′ 59″ North, 75° 10′ 1″ West).

The horses and mules were evaluated by two veterinarians who specialize in animal health and have worked as teachers in the area for more than 15 years. A preliminary pilot test was performed with a group of horses and mules (n = 30), belonging to the national police in the city of Manizales, to standardize the evaluation criteria for the behavioral and health variables included in the protocol.

Assessment of animal welfare indicators

A structured instrument was developed that assessed non-invasive indicators of animal welfare according to the protocol previously standardized by Pritchard et al. (6) and validated by the Brooke Hospital for Animals ("the Brooke"). Welfare indicators included measures of physical health and behavioral responses to human presence and contact. In general terms, and according to the guidelines proposed by Burn et al. (9), the following sequence was followed to take the measures included in the protocol: a) The animal's general alertness was assessed from a distance of at least 3 m for 10 s, before asking for the owner's informed consent, b) Once the informed consent was signed, the observer approached the animal at a normal pace, at a distance of 3 m, looking at the animal's neck or chest. The observer approached at an angle of about 20 degrees (not directly in front of the animal), then stopped 30 cm from the animal's head and recorded its response at the time they stopped, c) The observer walked alongside the animal from its head to its rear and back, keeping a distance of about 30 cm from its body, recording any signs of alertness, d) The observer gently placed their hand under the animal's chin, making just enough contact to support some weight, but not so much as to lift the head. If the animal moved its head away from the hand, the observer did not follow it. This was the first point of physical contact between the observer and the animal unless the animal itself had already initiated contact, d) Indicators related to physical health were then recorded, and finally, gait was assessed when the owner was asked to lead the animal for approximately six steps in a straight line away from the observer and then back toward the observer. The age of each animal was determined by dental chronometry examination by evaluating the incisors. The table with observed behaviors (Table 1) presents a brief description of the indicators evaluated in the animals. Pain behaviors were not explicitly included in the assessment.

Statistical analysis

Software Stata Version 13.0 (College Station, Texas, USA) was used for all the statistical analyses. Animals were considered experimental units. A descriptive analysis was made of the measurements, expressed in proportions of animals that presented the behavioral or health parameter observed in each species evaluated (equines and mules). Following the methodology proposed by Pritchard et al. (6), groups of observations belonging to similar categories were added to form aggregated scores for (a) lack of responsiveness to environmental/handling (general attitude + responsiveness to observer approach + responsiveness to observer walking down the side), (b) low body condition score (mucous membranes + coat condition + diarrhea + skin tent + heat stress), (c) lesions of skin and deeper tissues (firing lesions + swelling lesions + swelling of tendons/joints + deformed limbs + long hoof + hoof too short + sole surface abnormal + hoof horn quality) (6). The interactions between the different measures of behavior and health, as well as the interactions of these measures according to the age of the animals, were examined using a Chi-square test. Spearman rank correlation was then used to relate the measurements. A probability level of $P < 0.05$ was chosen as the limit for statistical significance in all tests, whereas probability levels of $P < 0.10$ and $P > 0.05$ were considered as a tendency.

Results

Characteristics of owners

Most owners were male ($n = 187$, 93.0%) and 7% ($n = 14$) were female. 80.5% ($n = 153$) had received primary school education and 19.5% ($n = 37$) had received high school education.

Characteristics of animals

Table 2 shows a description of the sex, age, and type of work performed by the evaluated animals, showing that the largest proportion of horses ($n = 160$, 80%) and mules were dedicated to Arrieria (muleteering) activity (cultivation of coffee, fruit trees, bananas, and others, as well as the transport of wood, food, work supplies for the farm and transport for their owners) and the rest ($n = 40$, 20%), as recreational horses (companionship and transport for their owners). The animals were distributed in different age groups, with a predominance of animals >5 years, in both horses and mules. The practice of castration of males was frequent (93.33%). Pregnant mares (84.0%) were taken to medical clinics to confirm gestational status by ultrasound examination and are not used for labor in the last third of pregnancy (Table 2).

Assessment of animal welfare indicators

No significant differences were observed in the behavioral and health indicators according to the type of work performed by the animals ($P > 0.05$). A high proportion of horses and mules presented a response to their environment with a general attitude of alertness (89.31 and 82.5%, respectively), without significant differences ($P > 0.05$). Horses and mules demonstrated friendly behavior in front of the evaluators (78.13 and 61.54%, respectively); apathetic or severely depressed behavior was low (10.7% vs. 17.5%) ($P > 0.05$). Statistically significant differences were found in the indicator of chin contact avoidance between horses and mules, with the negative reaction of the latter being greater ($P < 0.05$).

TABLE 1 Brief descriptions of the behavioral and physical measures taken as part of a working equine welfare assessment (4, 10).

Variable	Categorizations	Brief definition
General		
Age (y)	<5/5–15/>15	Assessed by observing the teeth
Sex	Stallion/gelding/mare/pregnant/mare	N/A
Work type	Arrieria	Agriculture activities, transport of goods by the mountains, transport of people
	Recreative	Transport of people
Behavior		
General alertness	Alert	Responding to surroundings, with active movement of the ears toward an existing stimulus. Eyes were usually wide open and head up unless sniffing or eating
	Apathetic or depressed	Passive response to surroundings, ears could be back, or lowered, eyes could be open, half or fully closed, head could be up
Observer approach	Response friendly	Movement of the head toward the observer with relaxed face and the eyes opened but not overly wide, forward turning of the ears
	Aggressive	Attempts to bite, rear, kick or strike with the foreleg
Walk-beside	No response	No obvious response
	Signs of attention	Signs of attention
Chin-contact	Accepts	Shows no response to chin-contact
	Avoids	Moves its head to avoid or reject contact, tense body position including upward holding of the head with tensed muscles and facial expression
General health		
Body condition	1–5 (including half-scores)	1, very thin; 5, very fat
Mucous membranes	Normal color	Examination of the gingival, labial, ocular, vaginal and penile mucosa: pink, moist and shiny
	Abnormal	Mucous membranes are pale, congestive, cyanotic or with endotoxemia halo, sticky or dry, and without brightness (dull)
Lesions at commissures of lips	Yes	Lesion of any kind including hair loss, healed lesion, scar
	No	Without lesion
Teeth missing	Yes	At least one tooth missing
	No	All teeth present
Molar hooks or sharp edges	Yes	Present
	No	Absent
Eyes	No abnormalities/abnormal	Healthy eyes
		At least one eye with wet eyelashes, discharge, redness, swelling, opacity, or injury
Coat staring	Yes	Matted
	No	Dry, uneven
Ectoparasites	Present Absent	Ticks, mites, bot eggs, lice, or lice eggs anywhere on the body
Fecal soiling	Present	Fecal soiling on inner thighs or hocks, or diarrhea observed during defecation
	Absent	
Heat stress	Present	Flared nostrils, increased respiratory rate, increased respiratory depth with head movement, apathy
	Absent	
Skin lesions		
General lesions	Present	Locations of lesions: breast, and shoulders, ears, forelegs, girth and belly, head, hindlegs, hindquarters, knees, lips, neck, point-of-hock, ribs, flank, tail and tail base, withers, and spine. Superficial/healed, broken skin (skin and immediate subcutaneous layers broken), or deep (visible muscle, tendon or bone)

(Continued)

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TABLE 1 (Continued)

Variable	Categorizations	Brief definition
	Absent (None<4 cm)	
Firing lesions	Yes	Cannon bone, suspensory ligament, flexor tendons, and fetlock joint is visible and distinct from each other in all four legs in one or more legs
	No	
Hoof horn quality	Normal/abnormal	Healthy/abnormalities
Hoof shape	Normal/abnormal	Healthy/abnormalities
Swelling of tendons/joints	Normal	Visual inspection of flexor tendons and fetlock joints; normal or swollen (suspensory ligament, flexor tendons and cannon bones indistinct)
	Abnormal	
Limb deformity	Normal	Lateral or flexural abnormalities of the limbs, excluding cow hocked conformation
	Abnormal	
Cow hocked conformation	Normal	A rotational change of the hindlimb
	Abnormal	
Hoof wall(s) conformation and quality	Normal	Visual inspection of hoof length and height (too long, too short)
	Abnormal	
Sole shape and structure	Normal	Round in horses, Healthy
	Abnormal	Abnormalities: asymmetrical shape, flat/convex or cracked sole, frog narrow, hard or absent, bars absent, or heels contracted
Gait	Normal	Normal
	Abnormal	Any reluctance to put weight on a limb and others (lameness or un-evenness, reluctance to put weight on limb, or uneven head-nodding or hip movement)
Lesions of skin and/or deeper tissues	Normal	Visual inspection of animals with full thickness skin or deeper lesions measuring at least 2 cm × 2 cm or 1 cm × 4 cm (superficial/healed, broken skin (skin and immediate subcutaneous layers broken), or deep (visible muscle, tendon or bone)
	Abnormal	

Eighty percent of the mules and 54.4% of the horses exhibited a healthy body condition score ($P < 0.05$), with a body condition score (BCS) of 3 or more on a scale of 1 to 5 (1, very thin; 5, very fat). The body condition index was positively and statistically significant ($P < 0.05$) and correlated with the presence of coat staring, ectoparasites, the condition of mucous membranes, and skin lesions in the corner of the mouth. Less than 15% of the animals had eye problems, limb deformities, and gait abnormalities. Injuries to the head, withers, spine, ribs/flank, hindquarters, and hind legs were observed in a frequency between 12.5 and 30.43% of the animals, the presence of coat staring and ectoparasites were more frequent in horses than in mules (Table 3). Lesions in the skin and/or deeper tissues showed a high prevalence in horses, particularly in the head, breast/shoulder, withers, spine, and ribs/flanks ($P < 0.05$). Additionally, a long hoof wall, abnormal hoof horn quality, and sole surface abnormal lesions were frequent in both horses and mules. The frequency of hoof abnormalities increased with the increasing age of the animals, and there were statistically significant differences in the frequency of long hoof wall and sole surface abnormal lesions ($P < 0.05$). The prevalence of skin lesions was higher in horses than in mules; animals with an age range between 5 and 15 years presented significant differences in the frequency of skin lesions located in the withers, spine, and ribs/flanks ($P < 0.05$) when compared to younger animals.

Weak correlations were found, but with statistically significant differences when aggregate behavioral parameters and aggregate health parameters were compared (R2 coefficient<0.5); in contrast, no significant correlation was observed between the behavior called "lack of response to the environment/handling" with low body condition score and skin lesions and deeper tissues ($P > 0.05$), as shown in Table 4.

Discussion

The evaluated animals may not necessarily represent the welfare status of all working equines in the coffee zone, nor in

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TABLE 2 Description of work type, sex and age group of 200 equids assessed in three departments of Caldas (Colombia).

	Specie					
	Horses $n = 160$ (80%)			Mule $n = 40$ (20%)		
	Total	Work type (%, *n*)		Total	Work type (%, *n*)	
		Arrieria	Recreative		Arrieria	Recreative
Sex						
Stallion	9.4 (15)	86.7 (13)	13.3 (2)	0 (0)	0 (0)	0 (0)
Gelding	37.3 (60)	93.3 (56)	6.7 (4)	55.0 (22)	95.5 (21)	4.5 (1)
Mare	37.3 (60)	80.0 (48)	20.0 (12)	45.0 (18)	94.4 (17)	5.6 (1)
Pregnant mare	16.0 (25)	84.0 (21)	12.0 (4)	0 (0)	0 (0)	0 (0)
Age group (years)						
<5	3.8 (6)	83.3 (5)	16.7 (1)	12.5 (5)	100.0 (5)	0 (0)
5-15	19.4 (31)	77.4 (24)	22.6 (7)	22.5 (9)	88.9 (8)	11.1 (1)
>5	76.6 (123)	87.9 (109)	12.1 (15)	65.0 (26)	96.1 (25)	3.9 (1)

Colombia, because management conditions may vary between the different geographical areas. Nevertheless, this study was carried out to have a baseline for the welfare status of working equines and mules, to identify causes of suffering, and to establish guidelines for the improvement of the well-being of the animals. Likewise, the implemented protocol is an easy, simple, and economical tool that can be adopted by owners to establish the indicator trends over time and evaluate the impact of the improvements that have been made.

Characteristics of owners

The owners and handlers of the horses and mules in this study were predominantly men. A similar situation is reported in Romania (3) but differs from that reported by Velázquez-Beltrán et al. (2) in the central region of Mexico, where differences according to gender were not observed. However, the activities in which equines are used were differentiated; women used donkeys to carry water and clothes, while men used a higher proportion of mules and horses for agricultural activities, as described in the coffee region of the present study. Additionally, a greater proportion of animal owners had completed their primary education, as has been described in Mexico (2). This aspect favors the viability of finding work in nearby urban centers in the region evaluated, which also reduces the need to migrate far from their village of origin and to make a living from agricultural activities (2).

Behavioral indicators

The behavioral observations used in this study have been used to establish an animal's responsiveness to the surrounding environment, and help to identify fear or aggression toward humans (6). Likewise, they allow for inferring human-equid interaction and the implications on the psychological state of the animals (11). Fear is considered a negative affective motivational state and in equine species this is a behavior that represents a serious risk of injury for handlers, resulting in a vicious cycle that increases the severity of restraint and fear (6). In this study, the most predominant behavior in response to the environment was the alertness of the animals. This is considered encouraging because some authors have suggested that general alertness or sensory attention behavior, which includes the reception of visual, auditory, olfactory, and sometimes tactile stimuli, is an important measure of animal welfare, representing an animal's interest or willingness to react positively to any sensory stimulation in the environment (10, 12). However, in this study, 10.7% of the horses and 15.5% of the mules were apathetic. Apathetic animals may require prioritization. Apathetic behavior is considered an indicator of poor animal welfare, possibly associated with problems related to disease, exhaustion, chronic pain, lethargy or depression, dehydration, and inconsistent rough handling, among others. (13). Additionally, chronic low back pain is associated with apathy or lack of sensory responsiveness in horses, according to a study by Rochais et al. (12), who evaluated 100 stable horses observed in their home environment. Therefore, it is important to educate owners in the identification of the causal factors of apathy in their animals, with special emphasis on encouraging consistent handling, humane training based on rewards, and the provision of appropriate food, water, and rest (13, 14).

In this study, horses and mules displayed friendly behavior in front of the evaluators, with horses showing a higher frequency of friendliness. Mules have been described as essential for pack work in difficult mountainous areas and superior to horses and donkeys, due to their better skills, endurance

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TABLE 3 Frequency (%) of behavior and health parameters of working horses and mules ($n = 200$).

Observations (%)	Species		*P*-value*
	Horses $n = 160$	**Mule** $n = 40$	
Behavior			
General attitude			
Alert	89.31 (142)	82.5 (33)	0.24
Apathic/severely depressed	10.69 (17)	17.5 (7)	
Response to observer approach[a]			
No response	3.13 (5)	7.69 (3)	0.11
Friendly approach	78.13 (125)	61.54 (24)	
Avoidance/aggression	17.5 (28)	30.77 (12)	
Walk downside[b]			
Response	84.47 (136)	65.0 (26)	0.10
No response	15.53 (24)	35.0 (14)	
Avoids chin contact[c]			
Accept	83.95 (161)	65.0 (26)	***<0.01***
Avoid	19.37 (39)	35.0 (14)	
Body condition score (scales 1-5)			
1	6.88 (11)	0 (0)	***<0.01***
2	38.75 (62)	20.0 (8)	
3	42.5 (68)	52.5 (21)	
4	11.88 (19)	27.5 (11)	
Health[c]			
Mucous membranes abnormal	10.0 (16)	7.69 (3)	0.12
Lesions at commissures of lips[d]	2.53 (4)	5.0 (2)	0.41
Teeth missing	8.18 (13)	2.56 (1)	*0.06*
Molar hooks or sharp edges	47.80 (76)	44.74 (17)	0.12
Eyes(s) abnormal[e]	3.13 (5)	7.5 (3)	0.20
Coat staring/matted/dry/uneven	27.95 (45)	5.0 (2)	***<0.01***
Ectoparasites	23.13 (37)	10.0 (4)	*0.06*
Diarrhea under tail	6.29 (10)	0 (0)	0.10
Skin tent (loss of elasticity)	16.77 (27)	7.5 (3)	0.14
Heat stress[f]	1.24 (2)	2.5 (1)	0.55
Firing lesions or scars[d]	62.50 (100)	47.5 (19)	*0.08*
Carpal lesions or scars[d]	11,80 (19)	20.0 (8)	0.17
Hock lesions or scars[d]	11.80 (19)	10.0 (4)	0.75
Swelling of tendons/joints	11.25 (18)	2.5 (1)	*0.09*
Limb deformity[g]	4.38 (7)	5.0 (2)	0.86
Cow hocked conformation	15.63 (25)	12.5 (5)	0.62
Hoof wall(s) too long	43.59 (68)	35.0 (14)	*0.09*
Hoof wall(s) too short	8.23 (13)	12.5 (5)	*0.09*
Hoof horn quality abnormal	48.73 (77)	42.5 (17)	0.48
Sole surface abnormal (RF)	34.18 (54)	31.58 (12)	0.84
Gait abnormal[h]	12.82 (20)	7.69 (3)	0.65
Lesions of skin and/or deeper tissues[i]			
Head	26.09 (42)	12.5 (5)	*0.07*
Ears	12.42 (20)	7.5 (3)	0.38
Neck	6.83 (11)	0 (0)	0.09
Breast/shoulder	16.88 (27)	15.0 (6)	0.77

(Continued)

TABLE 3 (Continued)

Observations (%)	Species		*P*-value*
	Horses $n = 160$	Mule $n = 40$	
Withers	35.4 (57)	12.5 (5)	***<0.01***
Spine	30.43 (49)	15.0 (6)	***0.05***
Girth	13.04 (21)	7.5 (3)	*0.09*
Belly	7.45 (12)	0 (0)	*0.07*
Ribs/flank	23.13 (37)	7.5 (3)	***0.03***
Hindquarters	17.5 (28)	7.5 (3)	0.11
Tail/tail base	9.38 (15)	7.5 (3)	0.13
Forelegs (except carpus)	11.8 (19)	2.5 (1)	*0.08*
Hindlegs (except hock)	15.53 (25)	2.5 (1)	***0.03***

[a]Response to the observer approaching the animal's head from 3 to 5 m away, at an angle of approximately 458 (more acute if the animal is wearing blinkers). Friendly approach: animal turns its head toward the observer. Avoidance/ aggression: animal does one or more of the following: turns head away, moves away, flattens ears, attempts to bite or kick.
[b]Response to observer walking downside of animal's body at a distance of 30 cm from its side, turning at the tail and walking back to head. Response: any acknowledgment of observer's presence, e.g., ear turn, head turn, move away, kick.
[c]Proportion of animals with signs of each condition.
[d]Proportion of animals with lesions of any kind including hair loss, healed lesion, scar.
[e] Proportion of animals with any abnormality of the eye including ocular discharge.
[f]Proportion of animals showing most or all of the following: flared nostrils, increased respiratory rate, increased respiratory depth with head movement, apathy.
[g]Proportion of animals showing lateral or flexural abnormalities of the limbs, excluding cow hocked conformation.
[h]Proportion of animals showing abnormalities of gait or overt lameness.
[i]Proportion of animals with full thickness skin or deeper lesions measuring at least 2 cm x 2 cm or 1 cm x 4 cm. Firing, tether, carpus, hock, and lip lesions scored previously were not included.
*Significance of difference in proportion between species by Chi-squared test. Bold values: $P < 0.05$.

capacity, better hoof quality, lower feed requirements, and greater working longevity (15). However, handlers perceive them as more aggressive and difficult to work with (16, 17). However, mules are creatures of habit and do not react well to changes in their daily routine and to contact with strangers. These animals tend to bond with humans after gaining trust; therefore, these results should be analyzed and interpreted with caution (18). Although the level of empathy of the owners with the horses and mules was not evaluated in this study, the *arrieria* (muleteer) culture transmitted from generation to generation in the Colombian coffee-growing region could have influenced a friendlier response of the animals to contact with two strangers, as there is close contact and a human-equine interaction that has been consolidated over years (2). Likewise, the friendly response to the observers may be related to the levels of empathy that the owners have toward the working equids, as the animals are often considered as family members, thus fostering a closer contact, understanding, and identification of the needs of their animals, as well as the building of routines and strong bonds with their handlers, as has been described in owners of working horses in Chile (19, 20), Brazil (21), and Italy (11). However, other studies conducted in Romania suggest that a particularly emotional relationship between the owner and his/her horse is not usually observed (3); these observed differences between studies may be related to specific geo-cultural factors, individual temperament traits of the animals, breed, interaction practices used by animal owners and handlers, reinforcement of occurrences that trigger positive affective states, and familiarity of the person conducting the test, among other aspects (3, 21).

In the walking alongside test, horses and mules were subjected to another common stimulus (the proximity of humans around them under usual working conditions). However, the frequency of animals with avoidance or fear behavior was higher than that found in the response to the observer approach test but lower than in the chin contact test. It has been suggested that this fear response may be associated with previous negative experiences, which are considered to be stable over time and across situations (9). In this study, the evaluation of the behavioral indicators was performed by two observers unknown to the animals, an aspect that could interfere with the obtained results. Research conducted in Romania evaluated the same behavioral indicators and found that the prevalence of the horses' behavioral responses presented significant differences when the tests were applied by the owner or by an unknown evaluator (3). Other studies with similar tests obtained very different results (1, 10, 15), possibly because of aspects such as: a) the cognitive ability of horses and mules to recognize familiar humans (18, 22), including facial recognition (23), and to remember specific experiences, especially bad ones (3), so it would be logical to obtain different reactions from the animals depending on the familiarity with the person performing the test; b) previous human-equine

TABLE 4 Correlations between aggregated behavior and health parameters of working horses and mules ($n = 200$).

Behavior and health parameters	Correlation coefficient	*P*
Lack of responsiveness to environment/handling[a]		
Low body condition score	−0.07	0.34
Lesions of skin and deeper tissues	−0.10	0.14
Systemic health abnormalities[b]	−0.11	**0.02**
Limb problems[c]	−0.22	***0.01***
Low body condition score		
Lesions of skin and deeper tissues	0.25	***<0.01***
Systemic health abnormalities[b]	0.47	***<0.01***
Limb problems[c]	0.26	***<0.01***
Lesions of skin and deeper tissues		
Systemic health abnormalities[b]	0.28	***<0.01***
Limb problems[c]	0.15	***0.03***
Limb problems[c]		
Systemic health abnormalities[b]	0.35	***<0.001***

[a]Aggregated score: general attitude + responsiveness to observer approach + responsiveness to observer walking down the side.
[b]Aggregated score: mucous membranes + coat condition + diarrhea + skin tent + heat stress.
[c]Aggregated score: firing lesions + swelling of tendons/joints + deformed limbs + hoof too long + hoof too short + sole surface abnormal (RF) + hoof horn quality.
Bold values: $P < 0.05$.

interactions, which when negative, can lead the animal to have excessive fear reactions, which can limit their use and make them dangerous for the conditions of the handlers (24) in which the test is performed (work routine, strange environment (10); d) individual temperament traits of the animals (3); e) the living environment (resources provided, tasks and demands of the work, climatic conditions, and geo-cultural characteristics, among others) (25); f) genetic characteristics and hybrid vigor, greater cognitive and endurance capacities of mules compared to horses are described (26), g) the experience and training of the observer for the evaluation of behavior and some health indicators such as body condition (27), among other aspects. In future studies, we consider it relevant to perform a comparative evaluation of the behavior of horses and mules in front of an unknown evaluator and the owner to control for possible measurement biases that could have occurred. However, having trained evaluators and a validated protocol were aspects that allowed us to obtain standardized information in this research.

Although no significant differences were found between the prevalence of the responses of horses and mules in three of the behavioral tests evaluated in this study (with the exception of chin contact avoidance), some authors suggest that mules, due to their hybrid nature resulting from the artificial crossbreeding of a mare (*Equus caballus*) and a donkey (*Equus asinus*), have probably acquired innate behaviors characteristic of each parental species; an aspect that is still under study due to a lack of knowledge (25). Mules do not have an evolutionary history in the natural environment as their parents do; therefore, it is even more difficult to infer the effects of domestication on the behavior of these hybrids, especially cognitive abilities and natural behaviors (28). Likewise, mules show more signs of avoidance or fear when an unfamiliar person makes repeated attempts to approach the animal for routine procedures or husbandry tasks (18), an aspect that has also been observed during approach tests conducted by known and unknown persons (6). In Colombia, mules are generally prized animals that are part of the coffee cultural landscape; likewise, Paso Fino mules are used for shows, trail rides, cultural tourism, and for sugarcane crops (29). The coffee culture and the attachment of muleteers toward their horses and mules, which are an important source of livelihood for them and their families, and are considered as family members by their owners (20), could have affected the low prevalence of negative behavioral responses toward unknown evaluators. On the other hand, the owners of the evaluated horses and mules voluntarily attended the veterinary days, which is an indication of their concern, commitment, and positive attitude toward their animals (20, 30). However, further studies that consider behavioral variations between horses and mules are required to develop improvements in the husbandry of these animals, focusing on their own needs and welfare conditions; as well as the awareness by owners of the particular body language and characteristics of mules, because unfortunately, their behavior has been misinterpreted by many in different countries, and harsh equipment, abusive tools, and cruel handling have been used to control them (18).

Body condition scoring in equids is very useful for its ability to detect welfare-relevant conditions, including undernutrition, overnutrition, metabolic disorders, laminitis, suboptimal management, and chronic coping difficulties (27). In this regard, there is a belief that mules are more robust than horses, an aspect that contributes to the lack of adequate care by handlers for their feeding and health needs, which can contribute to malnutrition, inadequate hydration, and, in most cases, overall poor animal welfare conditions (16). However, in this study, horses and mules presented healthy body conditions in a high proportion, this being higher in mules. When analyzing the proportion of adverse health indicators and body injuries due to overwork between horses and mules, the latter presented lower frequencies, therefore, better health conditions and management. These same results were described by Ali et al. (17) in Egypt when comparing the levels of animal welfare between donkeys and mules working in brick kilns, an aspect that revealed a greater adaptation of mules to adverse handling conditions.

Health problems

Oral diseases are one of the main clinical problems in horses and occupy the third place in the global veterinary diagnosis of this species, with dental abnormalities being responsible for most of the observed conditions, which go unnoticed, and in some occasions may produce pain (31); as is the case of pain caused by the use of headpieces and nosebands, which can press on the dental overgrowths (especially the vertical ridges of the upper teeth 06 and 07), causing trauma to the buccal side of the cheeks and lips, producing pain, biting problems and discomfort in the equine. Injuries induced by the bit or chiffney in the mandibular interdental space (bars of mouth) can occur due to excessive force with the bridle to direct the animals. In most cases, a superficial periostitis or sequestration of the mandibular cortex will occur (32). In the coffee-growing region evaluated, this equipment is not used on *arrieria* (muleteer) horses, only on workhorses that owners use for personal transport. In this study, abnormalities in the wear of premolar and molar teeth were prevalent, an aspect that coincides with previous studies done in Colombia (31, 33). This finding is very important from the point of view of the physical health and fitness for work of the animals, which can even compromise the performance or life of the animal, affect chewing, cause the presence of painful ulcers, periodontal disease, fractures, and loss of dental pieces, and cause deep infections of the alveolus (33). Considering the high prevalence of these problems, the implementation of a routine prophylactic program is recommended in every equine to prevent any form of malocclusion and correct overgrowths or excessive wear in time, and also to initiate treatment based on a correct diagnosis.

Skin lesions were frequently detected on the horses and mules in this study, with greater susceptibility in animals over five years old, an aspect that has also been described in working donkeys in Mexico (34, 35) and Ethiopia (36). Skin lesions (head, withers, spine, and ribs/flank) are associated with saddle and harness quality; these produce severe pain, especially those located in the withers, which can impact an animal's ability to work, particularly when loads are heavy (34, 36). In general, older animals may have a more prominent bone structure, resulting in increased contact that creates injuries from ill-fitting equipment or are the result of cumulative injuries over time. Older animals are more exposed to long working hours and carrying heavy loads during their working lives (35). Additionally, immune defense mechanisms are reduced with advancing age and sometimes their owners pay less attention to the treatment of their wounds (36).

Hoof condition is considered a general indicator of care and management of the animals by their owners. Hoof problems have been described as the most common cause of lameness in horses (27). In this study, the presence of horses and mules with long hoof walls and abnormal hoof horn quality occurred at a high frequency. The use of inadequate shoes and deficiencies in hoof trimming can lead to impaired balance, pressure on different parts of the hoof, stress on ligaments and/or tendons, and, finally, permanent gait disturbance (37). Lack of hoof care in animals may be related to poor management by the animal's owner and insufficient training strategies. Factors associated with poor owner management of their animals include economic constraints, lack of knowledge about management practices, the quality of human-horse interaction, owner attitudes, and insufficient owner commitment, among others (38, 39). Studies in India (37) and the United Kingdom demonstrated that long-term (2-year) participatory intervention projects involving animal owners, professionals, and handlers were successful in reducing limb problems and lameness in working horses, promoting adherence to treatment/care plans and positively impacting the quality of human-horse interaction. This style of intervention avoids confrontation and supports clients through a joint exploration of their beliefs, attitudes, and goals as a basis for supporting change in behavior (40).

Health problems and association with behavioral indicators

A low body condition in horses and mules was correlated with a lack of responsiveness to environment/handling, skin and deeper tissues lesions, systemic health abnormalities, and limb problems suggesting that working equids in poor health show an unresponsive behavioral profile, consistent with sickness, exhaustion, chronic pain, or depression-like states (9, 11). Likewise, it appears that equids with more severe physical problems enter a state of behavioral unresponsiveness, as is the case with animals in low body condition. The causes of low body condition are multifactorial and are likely to include malnutrition, overwork, parasitism, and disease, which could simultaneously cause behavioral unresponsiveness. Furthermore, apathy can lead to reduced appetite, as in sickness and depression, which in turn lowers body condition, as described by Burn et al. (10).

Added behavioral indicators in horses and mules in the coffee-growing area studied were correlated with the presence of systemic health abnormalities and limb problems. Studies in Afghanistan, Egypt, Ethiopia, Guatemala, India, Jordan, Kenya, Pakistan, and the Gambia suggest that equids with more severe physical problems enter a state of behavioral unresponsiveness because the animals' resources are being stretched to their limits and their fitness is compromised; likewise, as a "prey species", equids conserve their "energy" reserves as a survival strategy, even at the risk of not responding adequately to potentially threatening stimuli (9). This lack of response has been associated with different states of negative well-being, such as overwork exhaustion, chronic pain, apathy or depression, and general malaise (41, 42).

Conclusions

The low proportion of health and behavioral problems found in the study suggests that owners are concerned about the welfare of their working animals; however, it is important to emphasize that animals whose owners are not concerned about their medical care can be at risk of deteriorating health. The coffee culture and the attachment of the *arrieros* (muleteers) toward their horses and mules, which are an important source of livelihood for them and their families, could be factors that influenced these results. Therefore, independently of the level of schooling and economic possibilities of the owners, the results suggest that a good standard of working animal welfare can be achieved, because cultural factors and the desire and willingness to care for their animals are essential factors in favoring welfare. However, the presence of skin and deep tissue lesions, especially in horses, suggests that they are subjected to high workloads. Therefore, it is essential to train owners in aspects related to the importance of providing their equids with adequate rest periods to recover from work, and promote working hours that are in keeping with their health conditions. Collaborative interventions involving academia, animal owners and handlers could be the way forward for the shared exploration of knowledge, beliefs, attitudes and goals, as a basis for supporting behavioral change and positive human-equine interaction.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by Ethics Committee for Animal Experimentation (Act 24/06/2018, Activities with minimal risk) and the Human Ethics Committee (Act 15/06/2018) at the University of Caldas.

Author contributions

MR assisted with the conception, design of the experiment, preparation, data analysis, and preparation of manuscript. FM prepared the data for analysis and analyzed the data under the guidance of MR and JS. MR, JS, and FM contributed to interpretation of the results. MR and JS drafted and edited the manuscript. All authors read and approved the final manuscript.

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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.

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References

1. Pritchard JC. Animal traction and transport in the 21st century: getting the priorities right. *Vet J.* (2010) 186:271–4. doi: 10.1016/j.tvjl.2010.08.004

2. Velázquez-Beltrán LG, Sánchez-Vera E, Nava-Bernal EG, Arriaga-Jordán CM. The role of working equines to livelihoods in current day campesino hill-slope communities in central Mexico. *Trop Anim Health Prod.* (2011) 43:1623–32. doi: 10.1007/s11250-011-9881-6

3. Popescu S, Diugan E-A. The relationship between behavioral and other welfare indicators of working horses. *J Equine Vet Sci.* (2013) 33:1–12. doi: 10.1016/j.jevs.2012.04.001

4. Ali ABA, El Sayed MA, Matoock MY, Fouad MA, Heleski CR. A welfare assessment scoring system for working equids—A method for identifying at risk populations and for monitoring progress of welfare enhancement strategies (trialed in Egypt). *Appl Anim Behav Sci.* (2016) 176:52–62. doi: 10.1016/j.applanim.2015.12.001

5. Mekuria S, Mulachew M, Abebe R. Management practices and welfare problems encountered on working equids in Hawassa town, Southern Ethiopia. *J Vet Med Anim Heal.* (2013) 5:243–50. doi: 10.5897/JVMAH10.017

6. Pritchard JC, Lindberg AC, Main DCJ, Whay HR. Assessment of the welfare of working horses, mules and donkeys, using health and behaviour parameters. *Prev Vet Med.* (2005) 69:265–83. doi: 10.1016/j.prevetmed.2005.02.002

7. Mayorga CDA. Paisaje Cultural Cafetero, Patrimonio de la Humanidad. La cuestión del discurso patrimonial en contraste con el paisaje de la caficultura. *Territorios.* (2015) 32:35–59. doi: 10.12804/territ32.2015.02

8. Instituto Colombiano Agropecuario I. Reglamento que establece los requisitos para la expedición de licencias zoosanitarias de funcionamiento que autorizan las concentraciones de animiales y se señalan los requisitos sanitarios para los animales que participen en ellas. *D Of la República, Resolución 001634.* (2010). Available online at: https://www.ica.gov.co/getdoc/016f3c96-a458-4fa6-ae96-41d18b2221f5/requisitos-sanitarios-y-de-inocuidad-en-la-producc.aspx (accessed November 2, 2019).

9. Burn CC, Dennison TL, Whay HR. Environmental and demographic risk factors for poor welfare in working horses, donkeys and mules in developing countries. *Vet J.* (2010) 186:385–92. doi: 10.1016/j.tvjl.2009.09.016

10. Burn CC, Dennison TL, Whay HR. Relationships between behaviour and health in working horses, donkeys, and mules in developing countries. *Appl Anim Behav Sci.* (2010) 126:109–18. doi: 10.1016/j.applanim.2010.06.007

11. Dalla Costa E, Dai F, Murray LAM, Guazzetti S, Canali E, Minero M, et al. study on validity and reliability of on-farm tests to measure human–animal relationship in horses and donkeys. *Appl Anim Behav Sci.* (2015) 163:110–21. doi: 10.1016/j.applanim.2014.12.007

12. Rochais C, Fureix C, Lesimple C, Hausberger M. Lower attention to daily environment: a novel cue for detecting chronic horses' back pain? *Sci Rep.* (2016) 6:20117. doi: 10.1038/srep20117

13. Hall C, Goodwin D, Heleski C, Randle H, Waran N. Is there evidence of learned helplessness in horses? *J Appl Anim Welf Sci.* (2008) 11:249–66. doi: 10.1080/10888700802101130

14. Kumar N, Fisseha KK, Shishay N, Hagos Y. Welfare assessment of working donkeys in Mekelle city, Ethiopia. *Glob Vet.* (2014) 12:314–9. doi: 10.5829/idosi.gv.2014.12.03.82120

15. Lagos J, Rojas M, Rodrigues JB, Tadich T. Perceptions and attitudes towards mules in a group of soldiers. *Animals.* (2021) 11:1009. doi: 10.3390/ani11041009

16. Ali ABA, Matoock MY, Fouad MA, Heleski CR. Are mules or donkeys better adapted for Egyptian brick kiln work? (Until we can change the kilns) *J Vet Behav.* (2015) 10:158–65. doi: 10.1016/j.jveb.2014.12.003

17. Ali ABA, El Sayed MA, McLean AK, Heleski CR. Aggression in working mules and subsequent aggressive treatment by their handlers in Egyptian brick kilns—Cause or effect? *J Vet Behav.* (2019) 29:95–101. doi: 10.1016/j.jveb.2018.05.008

18. McLean AK, Navas González FJ, Canisso IF. Donkey and mule behavior. *Vet Clin North Am Equine Pract.* (2019) 35:575–88. doi: 10.1016/j.cveq.2019.08.010

19. Luna D, Vásquez R, Rojas M, Tadich T. Welfare status of working horses and owners' perceptions of their animals. *Animals.* (2017) 7:56. doi: 10.3390/ani7080056

20. Luna D, Vásquez R, Yáñez J. Tadich* T. The relationship between working horse welfare state and their owners' empathy level and perception of equine pain. *Anim Welf.* (2018) 27:115–23. doi: 10.7120/09627286.27.2.115

21. Hötzel MJ, Vieira MC, Leme DP. Exploring horse owners' and caretakers' perceptions of emotions and associated behaviors in horses. *J Vet Behav.* (2019) 29:18–24. doi: 10.1016/j.jveb.2018.10.002

22. Sankey C, Henry S, André N, Richard-Yris M-A, Hausberger M. Do horses have a concept of person? *PLoS ONE.* (2011) 6:e18331. doi: 10.1371/journal.pone.0018331

23. Stone SM. Human facial discrimination in horses: can they tell us apart? *Anim Cogn.* (2010) 13:51–61. doi: 10.1007/s10071-009-0244-x

24. Waiblinger S, Boivin X, Pedersen V, Tosi M-V, Janczak AM, Visser EK, et al. Assessing the human–animal relationship in farmed species: a critical review. *Appl Anim Behav Sci.* (2006) 101:185–242. doi: 10.1016/j.applanim.2006.02.001

25. Luz MPF, Maia CM, Gonçalvez HC, Puoli Filho JNP. Influence of workload and weather conditions on rolling behaviour of horses and mules. *Behav Processes.* (2021) 189:104433. doi: 10.1016/j.beproc.2021.104433

26. Proops L, Burden F, Osthaus B. Mule cognition: a case of hybrid vigour? *Anim Cogn.* (2009) 12:75–84. doi: 10.1007/s10071-008-0172-1

27. Hemsworth LM, Jongman E, Coleman GJ. Recreational horse welfare: The relationships between recreational horse owner attributes and recreational horse welfare. *Appl Anim Behav Sci.* (2015) 165:1–16. doi: 10.1016/j.applanim.2014.11.019

28. Burden F, Thiemann A. Donkeys are different. *J Equine Vet Sci.* (2015) 35:376–82. doi: 10.1016/j.jevs.2015.03.005

29. Mclean A. Comparing the physiological and biochemical parameters of mules and hinnies to horses and donkeys Amy K. McLean, PhD North Carolina State University Proceedings in the Donkey and Mule Welfare Symposuim, Hydra, Greece October (2015).

30. Haddy E, Brown J, Burden F, Raw Z, Kaminski J. Proops L. "What can we do to actually reach all these animals?" Evaluating approaches to improving working equid welfare. *PLoS ONE.* (2022) 17:e0273972. doi: 10.1371/journal.pone.0273972

31. Cruz Amaya JM, Sánchez V. JA, Vera Hernández LG. Caracterización y prevalencia de las enfermedades orales en el caballo criollo, departamento de Caldas, Colombia. *Rev Med Vet (Bogota).* (2012)39:73. doi: 10.19052/mv.73

32. Dixon PM, Dacre I. review of equine dental disorders. *Vet J.* (2005) 169:165–87. doi: 10.1016/j.tvjl.2004.03.022

33. Buitrago-Mejía J, Díaz-Cueto M, Suarez-Chica A, Cardona-Álvarez J. Distribución geográfica de la casuística clínica equina del servicio ambulatorio de grandes animales de la universidad de córdoba, colombia. *Rev Científica.* (2017) XXVII:270–81.

34. Burden FA, Du Toit N, Hernandez-Gil M, Prado-Ortiz O, Trawford AF. Selected health and management issues facing working donkeys presented for veterinary treatment in rural Mexico: some possible risk factors and potential intervention strategies. *Trop Anim Health Prod.* (2010) 42:597–605. doi: 10.1007/s11250-009-9462-0

35. Galindo F, de Aluja A, Cagigas R, Huerta LA, Tadich TA. Application of the hands-on donkey tool for assessing the welfare of working equids at Tuliman, Mexico. *J Appl Anim Welf Sci.* (2018) 21:93–100. doi: 10.1080/10888705.2017.1351365

36. Fsahaye S, Kumar N, Kebede E, Abebe N. Health and welfare assessment of working donkeys in and around Rama town, Tigray, Ethiopia. *Ethiop Vet J.* (2018) 22:26. doi: 10.4314/evj.v22i1.3

37. Reix CE, Dikshit AK, Hockenhull J, Parker RMA, Banerjee A, Burn CC, et al. Two-year participatory intervention project with owners to reduce lameness and limb abnormalities in working horses in Jaipur, India. *PLoS ONE.* (2015) 10:e0124342. doi: 10.1371/journal.pone.0124342

38. Pearson NY. *A Study of Horse Ownership and Management in Victoria*. Australia. (2003). Available online at: http://hdl.handle.net/11343/114868 (accessed September 20, 2022).

39. Paul H. Hemsworth GJC. Human-livestock interactions: The stockperson and the productivity and welfare of intensively farmed animals. 2nd ed. Wallingford UK: CABI International. (2011).

40. Lynden J, Hollands T, Ogden J, A. Farrier making every contact count: a microlevel analysis of farrier-client interaction for partnership working in managing a horse with laminitis. *J Equine Vet Sci.* (2020) 87:102924. doi: 10.1016/j.jevs.2020.102924

41. Ashley FH, Waterman-Pearson AE, Whay HR. Behavioural assessment of pain in horses and donkeys: application to clinical practice and future studies. *Equine Vet J.* (2010) 37:565–75. doi: 10.2746/042516405775314826

42. Pritchard JC, Burn CC, Barr ARS, Whay HR. Haematological and serum biochemical reference values for apparently healthy working horses in Pakistan. *Res Vet Sci.* (2009) 87:389–95. doi: 10.1016/j.rvsc.2009.05.003

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Animal welfare in Latin America: Trends and characteristics of scientific publications

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The present study constitutes a review of the scientific articles about animal welfare in terrestrial farmed animals, published in 19 countries of Latin America. The main objectives were to quantify and characterize articles produced between 1992 and 2021 in farm animals' welfare using "Web of Science [v.5.32]" and "CAB Abstracts" databases. A total of 663 articles were found for the period analyzed, which were mainly in English (87%). The countries with the most publications were Brazil (43%), México (25%), Chile (12%), Uruguay (10%), Colombia (4%) and Argentina (2%). Cattle was the farm species most considered in the publications (41%), and the studies addressed mostly the on-farm production stage (76%). There was a rapid increase in the number of articles published in the last 15 years, accounting for 95% of the publications. This could be related to the publication of welfare standards by the World Organization for Animal Health (WOAH) since 2005, the creation of the Collaborating Center for Animal Welfare and Sustainable Livestock Systems—Chile-Uruguay-México in 2009, a Regional Strategy of Animal Welfare prompted by the WOAH in 2012 and the inclusion of animal welfare in the veterinary curriculum. The fact that most articles were in English shows that Latin American researchers have somehow overcome the challenge of publishing in a non-native language and their research can be read/cited worldwide. However considerable gaps in scientific productivity were identified in comparison to European and North American countries. Scientific research concerning the livestock industry in Latin America faces new challenges arising from the need to move toward more sustainable production systems within the One Welfare and One Health frame.

KEYWORDS

animal welfare, animal behavior, scientific publications, research, farm animals, sustainability, Latin America

Introduction

Animal Welfare (AW) has become an increasingly important sociocultural, scientific, political, commercial and ethical issue of debate worldwide. The focus on the welfare of farm animals has not only affected intensive livestock production systems in various species, due to the restrictive conditions in which animals are kept and the husbandry practices they are submitted to increase productivity (1–3). AW also addresses other stressful stages for production animals that are of much public concern, like transport, marketing and pre-slaughter handling in general (4). Public concern is making the livestock industry move toward more AW friendly production and handling systems that must consider, animals' behavioral needs, sustainability, traceability and ethical quality of products of animal origin (5, 6).

Scientific research has played a fundamental role in detecting critical points for the welfare of farm animals (7). The role of scientists, veterinarians and other professionals dealing with livestock production has also been crucial for scientific progress, education and legislation on these issues (7, 8). In accordance with the One Health-One Welfare framework (9) that the World Organization for Animal Health (WOAH) is applying, results have shown that the need for a more humanitarian animal production should not be seen as a barrier or threat against livestock production systems but instead as an opportunity to achieve a more sustainable livestock production (5). By improving the health and productivity of animals the quantity and quality of animal products for the consumers may also increase (1, 4, 10).

The WOAH published the first AW standards/norms in 2005, and these have been further developed continuously up to present (11). The Region of the Americas of the WOAH has 31 member countries with a wide variety of food-producing animal species and husbandry systems (12). The member countries include USA and Canada, which are among the countries with the highest scientific productivity in AW (13, 14). However, by 2006 only a few Latin American countries had a system that could finance AW research and publications on the issue (15). In order to promote AW, enhance research under local conditions and also help implement the WOAH norms in this diverse region, a Collaborating Center for Animal Welfare and Sustainable Livestock Systems Chile-Uruguay-México (https://www.woah.org/es/que-ofrecemos/red-de-expertos/centros-colaboradores/#ui-id-3) was created in 2009 (16). Further on the Regional WOAH Office for the Americas published in 2012 a Regional AW Strategy (17) that was adopted by all member countries to enhance the implementation of AW norms. At the same time, this strategy aims to promote education and applied research in AW, according to the particular regional production conditions, in order to back new legislation and improve the welfare of production animals (17). In 2015, Glass et al. (18) determined the level of awareness and implementation of the American Regional Strategy. These authors reported the existence of working groups in AW in several countries, frequent organization of seminars and other training events, production of manuals of good practices in different species and other extension activities promoting AW, but the general implementation of the AW strategy was considered to be still in an initial phase. There has been an increasing development of new laws and regulations regarding animal protection in Latin American countries since the publication of the first AW standards in 2005 (10, 18).

The development of animal welfare science in Latin America has varied greatly from region to region and scientific research is limited to a few groups (12, 16). Scientific productivity is still considered to be low in Latin America compared to other regions like North America and the European Union. The published articles worldwide on AW and related areas, according to ISI Web of Knowledge and until 2016 (14), came mainly from the United States (33.48%), followed by UK, Germany and Canada; Latin American countries (Spanish and Portuguese speaking) contributed altogether with only 7.44% of all publications, with Brazil leading the list (4.47%). According to a more recent study by Freire and Nicol (13) the USA, UK and Germany have published most of the AW scientific articles in the last 30 years (period analyzed up to 2017), and Latin American countries are not mentioned because they hardly contributed to the total. None of the above-mentioned studies **analyzed** publications in terms of farm animals specifically, the type of species, stages of production or animal products that had been included in the studies.

It appears that Latin American countries have been developing new laws, local research and increasingly applying welfare standards that enhance the welfare of production animals (10), however there has been no quantitative measure of the possible progress in terms of scientific publications. In order to highlight trends in regional research in the area of farmed animals' welfare and get an overview of the scientific productivity, the objective of the present study was to determine the quantity of publications produced in total and per country on the welfare of terrestrial farmed animals in Latin America from 1992 to 2021, as well as identify the animal species and stages of production that have been considered so far in those publications.

Materials and methods

The methodology used in this study considered the following steps:

Definition of literature search strategies

Keywords (within the title, summary/abstract and author key words) that were related to "animal welfare" or "animal

behavior" in the area of "terrestrial production animals" corresponding to "Latin America" were selected. The search covered the years 1992 to 2021 in the CAB Abstracts (CAB) and Web of Science Core Collection (WoS) databases of the virtual library system of the Universidad Austral de Chile accessed *via* the FortClient programme. These databases were chosen because WoS had been used before in similar reviews on animal welfare publications (13, 14) and is considered worldwide an important database for scientific articles; CAB database was included because it has more journals indexed that accept articles in Spanish or Portuguese. The search and selected keywords were written according to the following strategy using Boolean search terms (AND, OR, *, ", $):

- CAB Abstract (animal* welfare* OR animal* behav*) AND (farm* animal* OR producti* animal* OR animal* producti* system* OR transport* OR stress* OR pain* OR stunn* OR bruis* OR handl* OR slaughter*).
- WoS (Web of Science) (animal welfare OR animal behav*) AND (farm* animal* OR producti* animal* OR animal* producti* system* OR transport* OR stress* OR pain* OR stunn* OR bruis* OR handl* OR slaughter*).

Article inclusion/exclusion criteria

All types of scientific articles (original articles, short communications and bibliographic reviews) published from 1992 to 2021 were included in the search (done in June 2022), with no language filter, considering journals in the areas of veterinary sciences, animal science, environmental sciences and food science in both databases.

From the list of 31 countries that appear as members of the WOAH in the Region of Americas, the name of each of the 19 countries in which Spanish or Portuguese is the main language (Latin American) was selected and included as a filter: Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Cuba, Ecuador, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Panama, Paraguay, Peru, Dominican Republic, Uruguay, and Venezuela. Once this was done for both databases, the first raw result was obtained and the references of these 846 articles were saved in a folder on the desktop, using the option to extract in RIS format file offered by CAB and WoS. The Mendeley Desktop program was then used to open the RIS format files and a matrix table with all the information was built using the Microsoft Excel Office Version 2021 program. Based on this selection, 143 publications were manually eliminated, because abstract revealed that the study did not actually correspond to the animal welfare or animal behavior areas (i.e., were only on productive traits), still referred to non-production animals (companion, sports, laboratory or zoo animals) or non-terrestrial species (fish and other aquatic species). Of the remaining 703 articles, most ($n = 507$) were found through WoS, and less through CAB ($n = 196$). Finally, 40 articles that were duplicated because they appeared both in WoS and CAB, were also eliminated. The resulting 663 articles (WoS plus CAB) were then manually categorized considering the following variables of interest:

Authors: first author.

Title: title of the article.

Journal: title of the journal in which the article was published and language of publications.

Year of publication: the year of each publication as appearing in the journal was registered.

Country of origin: the country of the first author was used; if the first author was not from Latin America as stated by institution of origin, then the country where the study was undertaken was used.

Species: cattle (beef, dairy, purpose not specified), sheep, goats, sheep and goats, poultry (layers, broilers, other), ruminants (in general, species not specified), pigs, equids (only if abstracts revealed a relation with production, farm work or slaughter, not sports), buffalos, South American camelids, rabbits, quails, chinchillas, guinea pigs, guinea fowl, wild boar, livestock in general (studies which refer to production animals in broad terms, without specifying any), surveys to people (farmers, transporters, slaughterhouse operators, consumers/public in general, students, veterinarians).

Production stage: The articles were categorized according to the analyzed/studied productive stage in the following groups: on-farm, during transport of livestock (loading, journey, unloading), pre-slaughter (when transport and slaughter were dealt with as one item), slaughter of livestock, livestock markets, other (surveys to people or general studies throughout all production stages). Further on, within the on-farm stage, articles were sub-classified according to its contents in: articles on AW and feeding/grazing behavior, nutrition and productive parameters; articles on AW and the environment (i.e., climate and housing systems, silvopastoral systems, thermal stress); articles dealing with stress, behavioral and physiological indicators of welfare; articles on AW and reproductive handling/techniques; articles concerning the human-animal relationship and handling/moving animals; articles on specific health issues in relation to AW; articles on painful husbandry practices.

Statistical analysis

Using the information collected in Microsoft Excel, tables were created from it to automatically count the information according to each variable. Descriptive statistics (numbers or percentages) were used and results are presented in graphs.

Results

A total of 663 published articles on farmed animals' welfare (FAW) were found for the 19 countries of Latin America

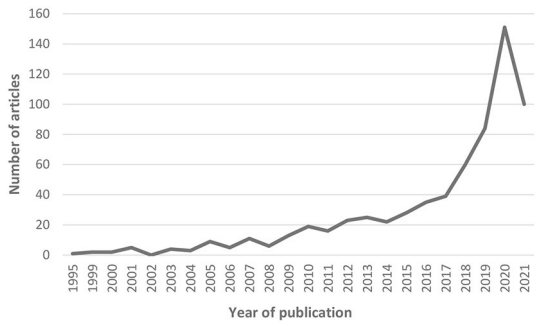

FIGURE 1
Publications on farm animal welfare in 19 countries of Latin America between 1992 and 2021 according to CAB and WoS data bases ($n = 663$).

between 1992 and 2021, considering the search through both databases. Regarding the distribution of the publications during the period analyzed, the earliest publication found was from 1995 by Caballero et al. in CAB (19) and there was an increase during time until 2020. A rapid increase in the total number of articles can be observed between years 2017 and 2020, where a peak of 151 articles was reached, whereas a decrease was observed in 2021 (Figure 1). Comparing the first 15-year period analyzed (1992–2006) and the last 15 years (2007–2021), 95% of all the publications was found in the latter period.

Considering the total of articles found (663), the countries with most publications on FAW during the period analyzed were Brazil (43%), México (25%), Chile (12%), Uruguay (10%), Colombia (4%), and Argentina (2%) (Figure 2). Ecuador, Costa Rica, Venezuela, Cuba and Perú (classified as "Others" in Figure 2) showed few publications that were also recent (2016–2018). No publications associated to FAW were found in Bolivia, El Salvador, Guatemala, Honduras, Nicaragua, Panamá, Paraguay, and Dominican Republic.

Figure 3 shows that publications on FAW in Latin America have dealt mainly with cattle (41%) and within these, more with beef (22%) than dairy cattle (19%). Studies on small ruminants were also common (22% including sheep, goats and South American camelids). Studies on pigs (12%) and poultry (9%, including broilers and layers) were less common. Among less conventional farm animal species, classified as "other species" (1%), there were articles on quails, wild boars, chinchillas, guinea fowl and guinea pigs. Five percent of articles dealt with surveys to people at different stages of production/education, aiming at their perception/appreciation/attitudes toward animal welfare.

When categorizing by stage within the production chain, the on-farm stage was the most considered, covering 76% of the articles (Figure 4). Within the on-farm stage, articles on the relationship between AW and feeding/grazing behavior, nutrition and productive parameters, were the most common (28%), followed by those on AW and the environment (i.e., climate and housing systems, silvopastoral systems, thermal stress, and 19%). Articles dealing with stress, behavioral and physiological indicators of welfare (15%) and those referring to AW and reproductive handling/techniques (12.5%) were also frequent. Articles concerning the human-animal relationship and handling/moving animals (7%), specific health issues in relation to AW (6%) and painful husbandry practices (4%) were less common. Articles dealing with the transport, pre-slaughter and slaughter stages, represented altogether 16% and covered mainly issues related to transport conditions, stunning procedures and meat quality (mainly bruises, carcass pH). Studies referring to livestock markets were uncommon. The category "across all stages" included the general studies on livestock covering the whole production chain.

The articles on FAW were published in a total of 155 journals. Most journals (119) were found to accept articles in English only, whereas a few (36) accept papers in Spanish and/or Portuguese (mostly English and Spanish, or English and Portuguese). Of the 663 articles found, 576 (87%) were published in English, whereas only 87 (13%) were published in Spanish/Portuguese. The journals where most articles on FAW were found were Animals ($n = 47$), Applied Animal Behavior Science ($n = 31$), Tropical Animal Health and Production ($n = 31$), Animal Production Science ($n = 26$), Livestock Science ($n = 24$), Animal ($n = 19$), Journal of Animal Behavior and Biometeorology ($n = 19$), Austral Journal of Veterinary Science ($n = 18$), Journal of Dairy Science ($n = 13$), and with 12 articles each were Semina:Ciencias Agrarias (Londrina) Brazil, Brazilian Journal Of Animal Science, Meat Science, Journal of Veterinary Behavior: Clinical Applications and Research, and Ciencia Rural, Brazil.

Discussion

This is a first and preliminary study giving an overview of the number and characteristics of the scientific articles on the welfare of terrestrial farm animals published in Latin America, covering a period of 30 years (between 1992 and 2021). The articles were analyzed in terms of number and year of publication, country of origin, animal species involved and production stages considered in the studies, as well as the journals and language of the publication, which will be discussed in the next sections.

Number of articles during the period analyzed

Our results agree with those of earlier bibliographic reviews (13, 14), showing that the productivity of scientific articles on AW in Latin American countries ($n = 663$) is in general

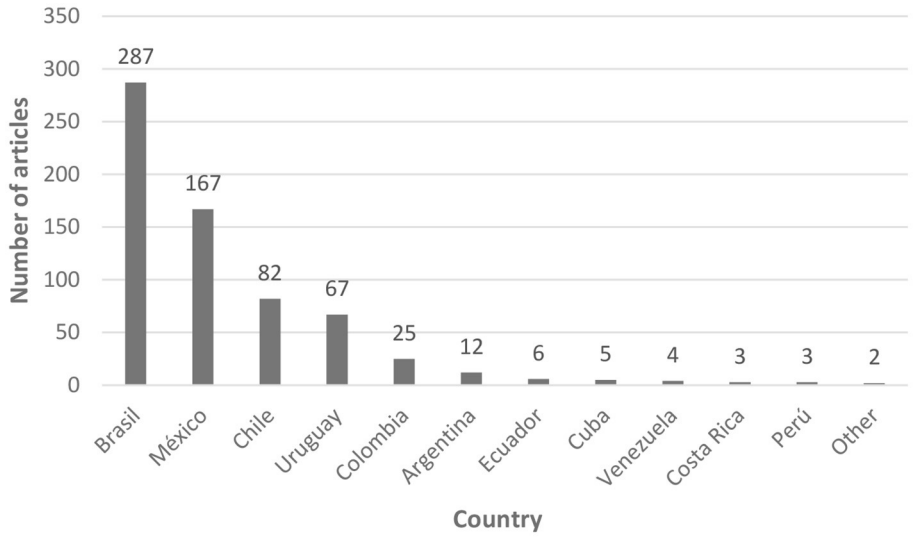

FIGURE 2
Latin American countries where scientific publications on farm animal welfare were produced between 1992 and 2021 ($n = 663$).

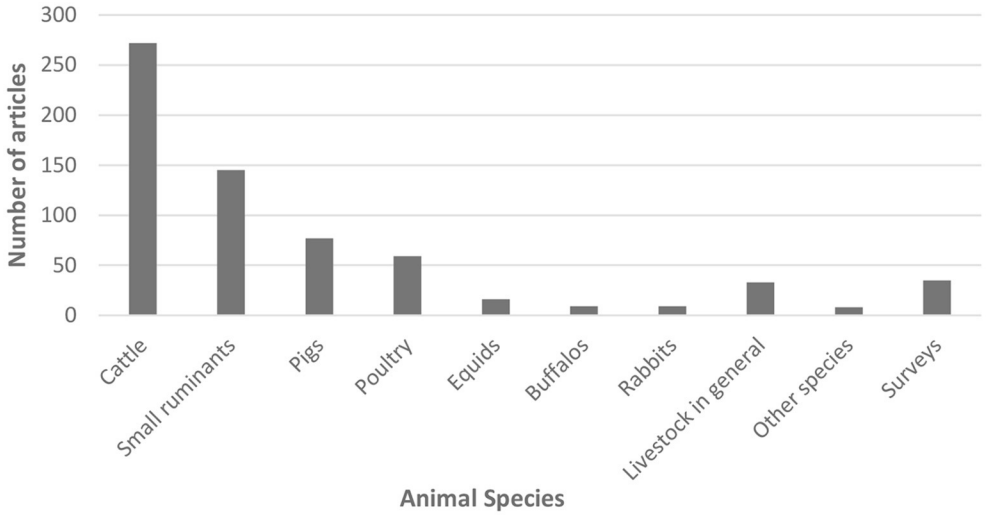

FIGURE 3
Animal species considered in the publications on farm animal welfare in Latin American countries between 1992 and 2021 ($n = 663$).

low compared to that of countries from North America and the European Union. Freire and Nicol (13) collected their data worldwide from the WoS, core collection-science citation index expanded (SCIEXPANDED 1968–2017), all languages and all types of documents and found between 10,349 and 15,614 publications on AW in general; however, they did not provide any numbers for publications originating specifically in Latin American countries. Mota-Rojas et al. (14) searched for publications in AW in general in Latin America plus Spain ("Iberoamerica"), using the Journal Citation Reports database in the Web of Knowledge and found 2,537 publications from Brazil, 669 from Mexico and 210 from Chile. In the present study we found 663 articles through the WoS and CAB databases and collected publications on the welfare of terrestrial farm animals only, greatly reducing the scope by excluding all publications on AW in aquatic animals, wild and zoo animals, sport horses, pets and laboratory animals. Considering that there is always a risk of bias in the selection of the key words and search words in this type of studies as indicated by Freire and Nicol (13), some articles might not have been found with the search

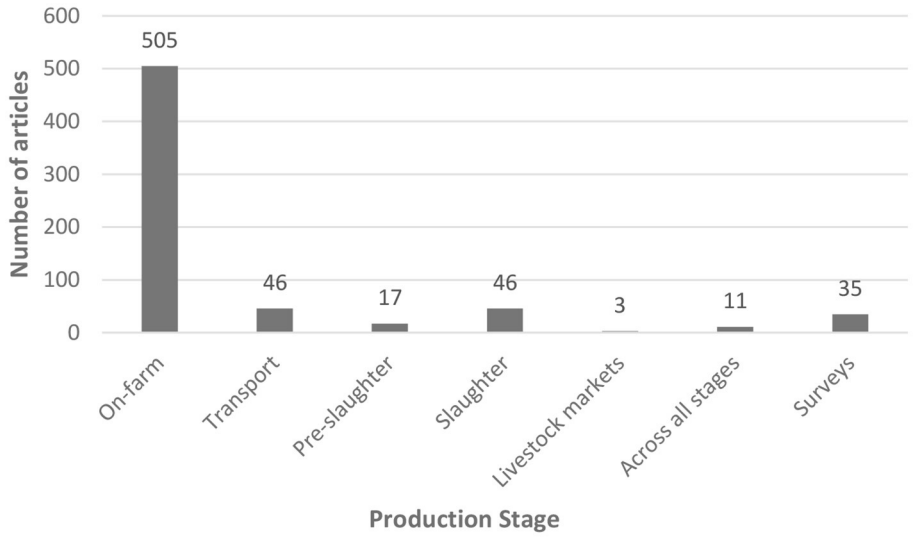

FIGURE 4
Production stages considered in the publications on farm animal welfare in Latin American countries between 1992 and 2021 ($n = 663$).

words used, which means that there is a risk that publications may be underestimated in our study. For instance, in the case of Chile and Uruguay, and perhaps other countries, the words "animal welfare" and "welfare" were not included in many of the older publications on AW, because there was some reticence from financing institutions to finance studies and research projects dealing directly with the issue, hence the authors avoided using the term specifically. More than actual numbers this study gives a preliminary insight on the trends of scientific articles on farm animal welfare (FAW) in Latin America.

Our study shows a large increase in the number of publications on FAW in Latin America throughout the time period analyzed, which coincides with the results of Freire and Nicol (13) for AW publications in general worldwide. However, in the case of Latin America, the increase is more recent, finding 95% of all articles published between 2007 and 2021, which indicates that animal welfare and behavior issues started developing in Latin America much later than in Europe and North America. The increasing interest in FAW in Latin America could be due to a worldwide trend observed toward animal welfare issues (20). This includes consumer and social pressure in general (21–23), and also the work of WOAH in publishing the AW standards (norms) for the first time in 2005 and promoting their implementation in member countries (11, 17). The need to include AW as an issue in international trade was also important in Latin America, where several of the main beef exporters are located (24, 25). For example, Brazil accounts for 23.5% of the world beef exports, Argentina 7.58%, Uruguay 3.81% and México 3.17% (26).

Another driver of research and publications in animal welfare could be related to education and regulation politics in the WOAH and the Latin American member countries. The implementation of animal welfare standards by Member Countries of the WOAH was determined in 2009 through a survey in 172 member countries (27). According to that survey, 66% of those countries identified veterinarians as the main responsible people behind the implementation of the standards and the development of legislation on AW in all countries. If veterinarians play a fundamental role in the study and research of AW and the implementation of politics in each country, then another important factor for the increase in publications may be that AW has been included by WOAH within the core curriculum in veterinary education (28). AW has been increasingly included as a compulsory subject in the curriculum of veterinarians and other professionals working in the livestock industry in Latin America since 2013 (29–31). In 2008 a 63% of the veterinary schools had at least one AW course included in the curriculum (32) whereas in 2016, in a sample of 100 out of the around 400 existing veterinary schools, this was true for 98% of them (31). The effects of the Regional AW Strategy of the Americas were only in an initial phase in 2015 (18), but it has certainly promoted further development of regulations in AW in many Latin American countries (10). Finally, due to the present economic and political importance of the subject of AW for many Latin American countries that are exporting animal products (24, 25), research financing institutions have also been prioritizing issues related to FAW in the last years.

Speaking from the experience of the authors, it was not easy to get funding for research in AW in the 90's as it was a new subject, sometimes regarded as a passing trend and thought

to be incompatible with production systems. The creation of the WOAH Collaborating Center for Animal Welfare and Sustainable Livestock Systems in this Region in 2009, has also prompted research in AW. It has disseminated results through the organization of large international conferences on AW in the three participating countries (in Chile 2009, 2018; in Uruguay 2012, 2022 and in México 2015). At these conferences young researchers from all Latin American countries have the opportunity to present their studies as well as meet colleagues and start collaborative research. The last conference gathered over 100 poster presentations and was held together with the regional International Society for Applied Ethology (ISAE) conference in 2018 (book of abstracts available at https://www.bienestaranimal.cl/wp-content/uploads/2019/07/Libro-de-Resumenes-BAISAE-2018.pdf), the most important scientific society on animal welfare science and active in Latin America since the early 90's (12, 16). Coincidently, the young researchers presenting their initial studies at our first meeting in 2009, are now heading their own research groups in AW in several Latin American countries and publishing their work.

The decrease in the number of published articles observed in 2021 could be related to the COVID-19 pandemic, but there are also other issues to be considered for the future of research and publications. An important factor is the large increase in the publication costs (APCs) imposed by most journals, which are difficult to be financed by many Latin American institutions and researchers, since they are usually higher than a researcher's monthly salary.

Main countries of origin of the publications on FAW

The main countries of origin of the articles on FAW were Brazil and México, which agrees with the findings of Mota-Rojas et al. (14) in his search for articles on animal welfare in Iberamerica. Freire and Nicol (13) also mention Brazil as the only visible Latin American country in their study of the scientific publications on AW worldwide, although they also mention that these articles have few citations. According to our study, Brazil, México, Chile, Colombia, Uruguay, and Argentina produced 96% of all articles on FAW. The leadership of Brazil in research and publications related to the livestock industry, in general, is probably due to its large geography within America, holding a similarly high human and cattle population (around 200 million each), and being the main meat exporter of the world (33). Besides beef exports, Brazil is also a main exporter of broiler and pork meat (34). México is also a large country in terms of human population and has a considerable cattle population (33 million) with a wide variety of husbandry systems. Another interesting factor may be that both countries also have many local journals that publish research findings in English and are WoS indexed, such as the Brazilian Journal of Animal Science, Ciencia Rural (Brazil), Revista Mexicana de Ciencias Pecuarias and Veterinaria México.

The WOAH Collaborating Center of AW and Sustainable Livestock Systems Chile-Uruguay-México has the objective of promoting AW in the region, hence it is not surprising that these countries were productive in terms of publications. There are groups of researchers on AW in each of these countries, which have networks or connections with researchers from most other Latin American countries (35). Accordingly, Universidad Nacional Autónoma de México, Universidad Austral de Chile and Universidad de la República in Uruguay, as part of the Collaborating Center have developed diverse strategies to promote the application of AW regulations and integrate AW within the production systems in Latin America (12, 16, 18, 35–37).

Only a few publications (appearing since 2016) were found in Cuba, Ecuador, Venezuela, Perú and Costa Rica, and none originated from Bolivia, El Salvador, Guatemala, Honduras, Nicaragua, Panamá, Paraguay and the Dominican Republic. This could be explained by the fact that several of these countries base their economies on activities different from livestock production (38, 39). However, Paraguay has a large cattle population and is a meat exporter and Bolivia has a similar situation. These countries may lack support for research from financing institutions, which prevents the development of research that could enhance their productive standards and improve the ethical quality of their products. According to the World Bank (40) the percentage of the gross domestic product (GDP) that Latin American countries invest in science and technology is still low, especially if compared to more developed countries. For example, Brazil invests the highest percentage in science and technology with 1.21%, followed by Uruguay (0.48%), Argentina (0.46%), Chile (0.34%), and México (0.3%), but countries as Perú, Bolivia, Paraguay are around 0.1% and Nicaragua only invests 0.03%. These percentages are much lower than the over 3% invested by the USA and Germany, and over 1.5% by Canada and the UK (40). The demands of countries from the European Union have encouraged countries like Brazil, Chile, Uruguay and Argentina to produce under higher welfare standards, and this could have been a driver for more research and then using evidence-based results for supporting changes in livestock handling and within the legislation (10, 25, 38, 41).

Characteristics of the publications on FAW: Species and stages of production

In terms of the characteristics of the publications and their contents, we found that these dealt mainly with cattle (41% of the articles) during the on-farm stage. Cattle is a farm species with high population in most Latin American countries (39) and

is also the most considered species worldwide in AW studies (13, 42). Our results show that the articles on FAW in Latin America dealt more with beef (22%) than dairy cattle (19%). This coincides with the fact that in Latin America we have several countries that are large beef producers and exporters, and therefore the interest in the welfare of farm animals and its relationship with meat quality was an initial driver for research (35, 43, 44). However, it differs from Freire and Nicol (13) who found that publications on AW worldwide dealt mainly with dairy cattle and were related to milk production and associated illnesses, such as lameness and mastitis. Recent studies in Chile and Brazil have also shown how cow welfare and productivity can be affected by lameness and mastitis (45–47) and a similar approach has been used looking at the welfare of dairy calves in relation to management, behavior and performance (48–51). Differences between studies and regions, are probably due to the fact that the dairy production systems in Europe, USA and Canada are more intensive and frequently combined with indoor housing, which often have worse welfare than extensively raised animals when we consider lack of comfort, insufficient space availability and fewer opportunities to perform natural behaviors (52). These characteristics pose a greater risk of welfare problems in more intensive systems and a greater need for research to find solutions. Although extensive production systems are generally regarded as more natural and welfare friendly, they may not provide livestock with enough shelter from inclement weather, food or water (extreme climate events), or protection from predators. This agrees with our findings on the topics most considered within the on-farm stage: 28% of the publications dealt with nutrition (feeding, grazing behavior in relation to productivity and AW) and 19% with comfort of the environment (climate, housing, thermal stress and others). Because beef and milk is produced mainly on large farms where animals are on pasture all year round, there is a growing interest in the welfare and productivity of dairy and beef cattle under heat stress and studies on the use of silvopastoral systems to mitigate heat stress and improve welfare have been undertaken recently (53–56). But the climate and the geography of Latin America is so variable, that the effects of cold and wet environments have also been considered recently in relation to welfare (57, 58).

At the beginning, Latin American publications dealt importantly with the welfare of meat producing species (cattle, sheep, pigs, broilers) which includes not only the stage of production on farm but also the transport, handling and slaughter stages (59–62). Hence earlier research focused on the relationship between AW and the quantity and quality of meat produced, which may be applied to all species producing meat for human consumption and is directly related to economic losses (43, 44, 63, 64). Several of the initial studies on long distance transport of cattle for slaughter in Chile and other countries in Latin America used productive (weight loss, carcass yield), health (mortality, lesions), stress (blood variables) and product quality (bruises and muscle pH) as AW indicators (44, 64–70). This was due mainly because countries like Brazil, Uruguay, Argentina and Chile have had the political and consumer pressure for including AW within their quality assurance schemes to be able to sell their meat to European countries, which are more demanding in terms of welfare. Today, AW has been recognized as part of the One Health/One Welfare concept (9) and an important issue related to the development of livestock productivity and sustainability (5, 71). Although research was initially more directed toward meat quality during the preslaughter stages and considered mainly productive indicators of welfare, it could be noticed in our review that more recent studies are increasingly using behavioral indicators of welfare that express not only negative but also positive emotional states and cognition of the animals (48, 50, 54, 72–75).

Studies analyzing compliance and impact of good handling practices on farm have also been undertaken in several countries and species (52, 76–78). Results show that there is still much research and publishing to do on species like poultry (layers and broilers) and swine, which are also exported as pork meat to Europe and Asia (39). Surprisingly, there are very few studies on species that one might think are related to smaller producers and important culturally, like South-American camelids or guinea pigs. We found only two articles on camelids (79, 80) and one in guinea pigs (81).

Animal suffering due to common husbandry practices during the on-farm stage of production like tail docking, dehorning and castration in various species has been an issue of debate among farmers, practitioners and the public in general. It was interesting to find several surveys in Latin America dealing with the perception of pain in animals by farmers and veterinary professionals, as well as studies on the effects of these husbandry practices directly on the expression of pain and stress in the animals (82–88). On the other hand, the tendency of people to increasingly consume more organic products and those produced under welfare friendly systems that avoid animal suffering as much as possible is growing (3, 20, 89). Several surveys on the issue were published during the last few years on the perception of Latin American consumers (90–95). There is a growing trend for livestock products to have a certification for animal welfare either from national or international certification bodies. Cage-free and free-range egg production systems in Latin America is a field of increasing interest, however, it appears that there is still a lack of knowledge related to the AW certifications and what these mean when it comes to consumers preferring one product over another (95). A recent survey by Cornish et al. (96) revealed that there is a better understanding and acceptance of certified products by consumers when they do not only get an AW seal, but also educational information on what parameters/indicators have been used to certify them and how the specific standards have been met.

Research in Latin America has expanded, moving from a Eurocentric perspective on the type of systems and problems studied to a wider spectrum of topics that in a way are the reflection of the diversity of agroecosystems and husbandry systems in the region. Efforts are still needed to promote and support more local research and the development of efficient policies based on sound science. In this sense, the WOAH has the potential to be a driver to strengthen networking with local actors, especially producers' organizations and industry in order to promote investment for a more strategic collaborative research on animal welfare.

Journals and language

Freire and Nicol (13) agreed on the need to close the gaps associated with language that are related to AW publications in Latin American countries. Scientific articles in Spanish or Portuguese have a reduced possibility to be read (and hence to be cited) because these will be shared mainly within Latin America and perhaps Spain and Portugal. Similarly, Sinclair et al. (97) reported that few articles on animal welfare in China have been translated into English and thus are unavailable for the global scientific community. This could create a misleading perception of a lack of interest about animal welfare in China. Our results show that 87% of the articles on FAW found in this search were published in journals that only accept articles in English. This shows that Latin American researchers have somehow overcome the difficulties of publishing in a non-native language, which used to create a significant barrier for publishing in high impact factor journals. Speaking from the experience of the authors, it is common that Latin American universities and institutions encourage their research staff to publish in English, because articles (and therefore also institutions) will get more visibility/readability and the likelihood of being cited increases. The fact that academic career is evaluated in terms of scientific productivity and impact of publications (10) and that in some universities researchers receive economic incentives for publications in high impact journals, has probably been an important driver in some Latin American countries for the noticeable increase in publications observed in the last 15 years and for publishing in English rather than in Spanish/Portuguese.

A strategy used by several of the most productive Latin American authors to facilitate publishing in English and increase productivity and readability of their articles has been to work and publish in collaboration with North American and European English-speaking researchers who work in the same fields within FAW. Although in the present study we did not quantify how many articles have been coauthored with researchers/institutes from regions outside of Latin America, some examples of these joint publications are von Keyserlingk and Hötzel (2), Gallo et al. (59–61), Huertas et al. (98), Broom et al. (99), Tadich et al. (100), Strappini et al. (101), Miranda de la Lama et al. (63). This is the result of the interaction between key international researchers in FAW, many of whom have been doctoral or master's thesis supervisors of younger Latin American researchers or have met at international conferences and then been invited to visit and speak at conferences in Latin America. This interaction between researchers from other regions has facilitated collaborative research and also publishing in English. Collaborative networking among Latin American researchers in FAW has also been successful and authors of different countries within the region were identified to be linked through co-authoring publications (29, 32, 35, 43, 74, 102–105). Further analysis should follow in order to provide quantitative data regarding the groups of researchers working in specific topics of farm animal welfare, the main authors and their connections within the region and with other regions, because this could help enhancing animal welfare development in Latin America.

Conclusions

The number of publications on farm animal welfare in Latin America is still low compared to more developed regions of the world, however, an important increase in articles was found during the last 15 years. This could be related to the implementation of the WOAH standards for animal welfare worldwide since 2005, but also to political reasons that have included animal welfare as an issue in international trade and the consequent interest of Latin American countries to increase research in the area in order to meet certain welfare standards. In fact, the six countries (Brasil, México, Chile, Colombia, Uruguay and Argentina) that produced 96% of all articles on farm animal welfare are important meat exporters. This coincides with the fact that most publications dealt with meat production species like cattle, sheep, pigs and poultry, during the on-farm production stage.

Another driver for the increase in publications could have been the inclusion of animal welfare within the veterinary curriculum, which opened new areas of research for the students, as well as universities prompting their staff to publish in high impact journals. The fact that most of the articles on farm animal welfare in Latin America were in journals that publish in English shows that Latin American researchers have somehow overcome the language problem and their research can be read/cited worldwide. Further analysis of the publications on farm animal welfare in Latin America should include citations of the articles, as well as identifying research groups/authors and networking, in order to provide information on the impact research in this region may have worldwide.

Author contributions

CG was responsible for general supervision and writing the first draft. LV was responsible for the search and initial analysis, TT was responsible for analysis and descriptive statistics. CG,

TT, SH, and FG contributed to the writing and discussion of the manuscript in its final version. All authors contributed to the article and approved the submitted version.

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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.

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References

1. Molento CFM, Bond GB. Production and animal welfare; ethical and technical aspects of bovine production. *Ciênc Vet Tróp*. (2008) 11(Supl 1):36–55.

2. Von Keyserlingk M, Hötzel MJ. The ticking clock: addressing farm animal welfare in emerging countries. *J Agric Environ Ethics*. (2015) 28:179–95. doi: 10.1007/s10806-014-9518-7

3. Shields S, Shapiro P, Rowan A. A decade of progress towards ending the intensive confinement of farm animals in the United States. *Animals*. (2017) 7:40. doi: 10.3390/ani7050040

4. Broom DM. Animal welfare: an aspect of care, sustainability, and food quality required by the public. *J Vet Med Educ*. (2010) 37:83–8. doi: 10.3138/jvme.37.1.83

5. Keeling L, Tunón H, Olmos Antillón G, Berg C, Jone M, Stuard L, Swanson J, Wallenbec A, Winckler C, Blokhuis H. Animal welfare and the United Nations sustainable development goals. *Front Vet Sci*. (2019) 6:336. doi: 10.3389/fvets.2019.00336

6. Olmos-Antillón G, Tunón H, de Oliveira D, Jones M, Wallenbeck A, Swanson J, et al. Animal welfare and the United Nations' sustainable development goals—broadening students' perspectives. *Sustainability*. (2021) 13:3328. doi: 10.3390/su13063328

7. Manteca X. Tendencias de la investigación científica en Bienestar Animal. In: González G, Stuardo L, Benavides P, Villalobos P, editors. *Actas del Seminario La Institucionalización del Bienestar Animal, un Requisito para su Desarrollo Normativo, Científico y Productivo*. Santiago de Chile: Salvat Impresores (2004), 29–44.

8. Ventura B, Weary D, Giovanetti A, Von Keyserlingk M. Veterinary perspectives on cattle welfare challenges and solutions. *Livest Sci*. (2016) 193:95–102. doi: 10.1016/j.livsci.2016.10.004

9. García Pinillos R, Appleby MC, Manteca X, Scott-Park F, Smith C, Velarde A. One welfare-a platform for improving human and animal welfare. *Vet Rec*. (2016) 179:412–3. doi: 10.1136/vr.i5470

10. Gallo C, Tadich T. Perspective from Latin America. In: Mench J, editors. *Advances in Agricultural Animal Welfare. Science and Practice*. Duxford: Woodhead Publishing (2017), 197–218. doi: 10.1016/B978-0-08-101215-4.00011-0

11. WOAH (World Organization for Animal Health). *Terrestrial Code. 7, Animal Welfare. Ed*. Paris: OIE (2022). Available online at: https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/?id=169&L=1&htmfile=titre_1.7.htm (accessed August, 2022).

12. Galindo F, Huertas SM, Gallo C. Recomendaciones de la OIE para la enseñanza de la Etología y el Bienestar Animal en el contexto de la estrategia regional para las Américas. In: Taylor-Preciado JJ, editors. *Inclusión de temas de Bienestar Animal en planes de estudio de Medicina Veterinaria en Latinoamérica*. Guadalajara: Amateditorial S.A. (2016), 9–16.

13. Freire R, Nicol CJ. A bibliometric analysis of past and emergent trends in animal welfare. *Anim Welfare*. (2019) 28:465–85. doi: 10.7120/09627286.28.4.465

14. Mota-Rojas D, Taylor-Preciado J, Ramírez-Necoechea R, Mora-Medina P. Bienestar Animal en Iberoamérica: seguimiento de artículos científicos. In: Taylor-Preciado JJ, editors. *Inclusión de temas de Bienestar Animal en planes de estudio de Medicina Veterinaria en Latinoamérica*. Guadalajara: Amateditorial S.A. (2016), 31–9.

15. Gallo C. Animal Welfare in the Americas. In: *Proceedings of Technical Items Presented to the International Committee and Regional OIE Commissions, Florianopolis, Brazil 2016*. Paris: World Organization for Animal Health (2006), 159–66.

16. Galindo F, Tadich T, Ungerfeld R, Hotzel MJ, Miguel-Pacheco J. The development of applied ethology in Latin America. In: Brown J, and Seddon Y, editors. *Animals and us: 50 years and more of applied ethology, ISAE 50th Anniversary, 1st Edn*. Netherlands: Wageningen Academic Publishers (2016), 211–25. doi: 10.3920/978-90-8686-828-5_10

17. WOAH (World Organization for Animal Health). *Regional Animal Welfare Strategy for the Americas*. Paris: WOAH (World Organization for Animal Health) (2020). Available online at: https://rr-americas.oie.int/wp-content/uploads/2020/01/estrategia-regional-reporte-final-barbados_esp.pdf

18. Glass E, Kahn S, Arroyo Kuribreña M. Awareness and implementation of the regional animal welfare strategy for the Americas: a questionnaire. *Rev Sci Tech Off Int Epiz*. (2015) 34:673–88. doi: 10.20506/rst.34.3.2388

19. Caballero C, Ocampo C, Sumano L. A review of the use of recombinant bovine somatotropin during conditions of heat stress in dairy cattle. *Técnica Pec México*. (1995) 33:168–77.

20. Verbeke W. Stakeholder, citizen and consumer interests in farm animal welfare. *Anim Welfare*. (2009) 18:325–33.

21. Santurtún Oliveros E, Tapia Pérez G, González Rebeles C, Galindo F. Consumer attitudes and perceptions towards sustainable animal production attributes in Mexico City. *Veterinaria México OA*. (2012) 43:87–101. Available online at: https://www.scielo.org.mx/pdf/vetmex/v43n2/v43n2a1.pdf

22. Lama GC, Estévez-Moreno LX, Sepúlveda WS, Estrada-Chavero MC, Rayas-Amor AA, Villarroel M, et al. Mexican consumers' perceptions and attitudes towards farm animal welfare and willingness to pay for welfare friendly meat products. *Meat Sci*. (2017) 125:106–13. doi: 10.1016/j.meatsci.2016.12.001

23. Rossi Borges JA, de Faria Domingues CH, Ribeiro Caldara F, da Rosa NP, Senger I, Gomes Freire Guidolin D. Identifying the factors impacting on farmers' intention to adopt animal friendly practices. *Prevent Vet Med*. (2019) 170:104718. doi: 10.1016/j.prevetmed.2019.104718

24. Thiermann A, Babcock S. Animal welfare and international trade. *Rev Sci Tech Int Off Epiz*. (2005) 24:747–55. doi: 10.20506/rst.24.2.1600

25. González G, Stuardo L, Benavides P, Villalobos P. *Actas del Seminario La Institucionalización del Bienestar Animal, un Requisito para su Desarrollo Normativo, Científico y Productivo*. Santiago de Chile: Salvat Impresores (2004), 15–6.

26. USDA. *Ranking of Countries that Export the Most Beef*. Washington, DC: USDA. Available online at: https://beef2live.com/story-world-beef-exports-ranking-countries-0-106903-printversion

27. Stafford K, Mellor D. The implementation of animal welfare standards by Member Countries of the World Organisation for Animal Health (OIE): analysis of an OIE questionnaire. *Rev Sci Tech Off Int Epiz.* (2009) 28:1143–64. doi: 10.20506/rst.28.3.1959

28. WOAH, World Organisation for Animal Health. *(OIE [Office International des Épizooties]) Veterinary Education Core Curriculum OIE Guidelines*. Paris: WOAH (World Organization for Animal Health) (2013).

29. Mota-Rojas D, Orihuela A, Strappini A, Cajiao M, Agüera E, Mora-Medina P, et al. Teaching animal welfare in veterinary schools in Latin America. *Int J Vet Sci Med.* (2018) 6:131–40. doi: 10.1016/j.ijvsm.2018.07.003

30. Hewson C, Baranyiová E, Broom DM, Cockram, MS, Galindo F, et al. Approaches to teaching animal welfare at thirteen veterinary schools in Europe, North America and South America. *J Vet Med Educ.* (2005) 32:422–37. doi: 10.3138/jvme.32.4.422

31. Taylor-Preciado JJ. Inclusión de temas de bienestar animal en planes de estudio de Medicina Veterinaria en Latinoamérica. In: Amateditorial SA, editor. *Asociación Panamericana de Ciencias Veterinarias (PANVET), Federación Panamericana de Facultades y Escuelas de Ciencias Veterinarias*. Guadalajara: FAO y Universidad de Guadalajara (2016), 153.

32. Tadich N, Molento C, Gallo C. Teaching animal welfare in some veterinary schools in Latin America. *J Vet Med Educ.* (2010) 37:69–73. doi: 10.3138/jvme.37.1.69

33. IBGE (Instituto Brasileiro de Geografia e Estatística). *Pesquisa Trimestral do Abate de Animais*. Brazil: IBGE (2021). Available online at: https://www.ibge.gov.br/estatisticas/economicas/agricultura-epecuaria/9203-pesquisas-trimestrais-do-abate-de-animais.html?=&t=o-que-e (accessed September 28, 2021).

34. Juárez C. *Brasil, líder en la exportación de carne bovina y de pollo*. Sterling, VA: The Food Tech (2021). Available online at: https://thefoodtech.com/industria-alimentaria-hoy/brasil-lider-en-la-exportacion-de-carne-bovina-y-de-pollo (accessed September 12, 2021).

35. Huertas S, Gallo C, Galindo F. Drivers of animal welfare policy in the Americas. *Rev Sci Tech Off Int Epiz.* (2014) 33:67–76. doi: 10.20506/rst.33.1.2264

36. Rojas H, Stuardo L, Benavides D. Animal health policies and practices in the Americas: preliminary study. *Rev Sci Tech Int Off Épiz.* (2005), 24:549–65. doi: 10.20506/rst.24.2.1589

37. Tadich T, Stuardo L. Strategies for improving the welfare of working equids in the Americas: a Chilean example. *Rev Sci Tech Int Off Epiz.* (2014) 33:203–11. doi: 10.20506/rst.33.1.2271

38. Labraga J. *Exportaciones de Carne Bovina del MERCOSUR: Una Cuantificación de Los Efectos Comerciales de Medidas Sanitarias Nuevas y Tradicionales*. Julio: Instituto para la Integración de América Latina y el Caribe (INTAL) IDB-TN-1046 (2016). Available online at: https://publications.iadb.org/publications/spanish/document/Exportaciones-de-carne-bovina-del-MERCOSUR-Una-cuantificaci%C3%B3n-de-los-efectos-comerciales-de-medidas-sanitarias-nuevas-y-tradicionales.pdf

39. FAO. *Situación del Mercado: Carne*. Rome: FAO (2017). Available online at: https://www.fao.org/3/BT089s/BT089s.pdf (accessed June 4, 2021).

40. World Bank. *Research and Development Expenditure (% of GDP)*. Washington, DC: World Bank (2022). Available online at: https://data.worldbank.org/indicator/GB.XPD.RSDV.GD.ZS (accessed July 7, 2022).

41. Bowles D, Paskin R, Gutiérrez M, Kasterine A. Animal welfare and developing countries: opportunities for trade in high-welfare products from developing countries. *Rev Sci Tech Off Int Epiz.* (2005) 24:783–90. doi: 10.20506/rst.24.2.1610

42. Von Keyserlingk M, Weary, D. A 100-year review: animal welfare in the journal of dairy science—the first 100 years. *J Dairy Sci.* (2017) 100:10432–44. doi: 10.3168/jds.2017-13298

43. Paranhos Da Costa M, Huertas S, Gallo C, Dalla Costa O. Strategies to promote farm animal welfare in Latin America and their effects on carcass and meat quality traits. *Meat Sci.* (2012) 92:221–6. doi: 10.1016/j.meatsci.2012.03.005

44. Gallo CB, Huertas SM. Main animal welfare problems in ruminant livestock during preslaughter operations: a South-American view. *Animal.* (2016) 10:342–8. doi: 10.1017/S1751731115001597

45. Tadich N, Hettich E, Schaik G. Prevalence of lameness in cows from 50 dairy herds in southern Chile. *Arch Med Vet.* (2005) 37:29–36. doi: 10.4067/S0301-732X2005000100005

46. Green L, Borkert J, Monti G, Tadich N. Associations between lesion-specific lameness and the milk yield of 1,635 dairy cows from seven herds in the Xth region of Chile and implications for management of lame dairy cows worldwide. *Anim Welfare.* (2010) 19:419–27.

47. Bran J, Costa J, Keyserlingk M, Hötzel M. Factors associated with lameness prevalence in lactating cows housed in freestall and compost-bedded pack dairy farms in southern Brazil. *Prev Vet Med.* (2019) 172:104773. doi: 10.1016/j.prevetmed.2019.104773

48. Hoetzel MJ, Ungerfeld R, Quintans G. Behavioral responses of 6-month-old beef calves prevented from suckling: influence of dam's milk yield. *Anim Prod Sci.* (2010) 50:909–15. doi: 10.1071/AN09136

49. Hoetzel MJ, Longo C, Balcao LF, Cardoso CS, Costa JHC. A survey of management practices that influence performance and welfare of dairy calves reared in southern Brazil. *PLoS ONE.* (2014) 9:e114995. doi: 10.1371/journal.pone.0114995

50. Calderón-Amor JA, Beaver A, von Keyserlingk MAG, Gallo C. Calf- and herd-level factors associated with dairy-calf reactivity. *J Dairy Sci.* (2020) 103:4606–17. doi: 10.3168/jds.2019-16878

51. Calderón-Amor J, Gallo C. Dairy calf welfare and factors associated with diarrhea and respiratory disease among Chilean dairy farms. *Animals.* (2020) 10:1115. doi: 10.3390/ani10071115

52. Hernández A, Estrada-König D, Romero-Zúñiga JJ, Galina CS, Berg C, Rojas-Gonzales M, et al. Implementation of the welfare quality protocol in dairy farms raised on extensive, semi-intensive and intensive systems in Costa Rica. *J Anim Behav Biometeorol.* (2017) 5:132–8. doi: 10.31893/2318-1265jabb.v5n4p132-138

53. Mancera KF, Zarza H, López de Buen L, Carasco García AA, Montiel Palacios F, Galindo F. Integrating links between tree coverage and cattle welfare in silvopastoril systems evaluation. *Agron Sust Develop.* (2018) 38:19. doi: 10.1007/s13593-018-0497-3

54. Améndola L, Solorio FJ, Ku-Vera JC, Améndola-Massioti RD, Zarza H, Mancera KF, et al. A pilot study on the foraging behaviour of heifers in intensive silvopastoral and monoculture systems in the tropics. *Animal.* (2019) 3:606–16. doi: 10.1017/S1751731118001532

55. Deniz M, Schmitt A, Hötzel M, de Sousa K, Pinheiro L, Sinisgalli P. Microclimate and pasture area preferences by dairy cows under high biodiversity silvopastoral system in Southern Brazil. *Int J Biometeorol.* (2020) 64:1877–87. doi: 10.1007/s00484-020-01975-0

56. Deniz M, de Sousa KT, Moro MF, do Vale MM, Dittrich JR, Pinheiro Machado Filho LC, et al. Social hierarchy influences dairy cows' use of shade in a silvopastoral system under intensive rotational grazing. *Appl Anim Beh Sci.* (2021) 244:105467. doi: 10.1016/j.applanim.2021.105467

57. Cartes D, Strappini A, Sepulveda-Varas P. Provision of shelter during the prepartum period: effects on behavior, blood analytes, and health status in dairy cows in winter. *J Dairy Sci.* (2021) 104:3508–21. doi: 10.3168/jds.2020-19439

58. Matamala F, Strappini A, Sepúlveda-Varas P. Dairy cow behaviour around calving: its relationship with management practices and environmental conditions. *Aust J Vet Sci.* (2021) 53:0719–8132. doi: 10.4067/S0719-81322021000100009

59. Gallo C, Teuber C, Cartes M, Uribe H, Grandin T. Mejoras en la insensibilización de bovinos con pistola neumática de proyectil retenido tras cambios de equipamiento y capacitación del personal. *Arch Med Vet.* (2003) 35:4. doi: 10.4067/S0301-732X2003000200004

60. Gallo C, Lizondo G, Knowles T. The effects of journey and lairage time on steers transported to slaughter in Chile. *Vet Rec.* (2003) 152:361–4. doi: 10.1136/vr.152.12.361

61. Gallo C, Warriss P, Knowles T, Negrón R, Valdés A, Mencarini I. Densidades de carga utilizadas para el transporte de bovinos destinados a matadero en Chile. *Arch Med Vet.* (2005) 37:155–9. doi: 10.4067/S0301-732X2005000200010

62. Miranda-de la Lama G, Leyva I, Barrera-Serrano A, Pérez-Linares C, Sánchez-López E, María G, et al. Assessment of cattle welfare at a commercial slaughter plant in the northwest of México. *Trop Anim Health Prod.* (2012) 44:497–504. doi: 10.1007/s11250-011-9925-y

63. Miranda de la Lama G, Gonzales C, Gutiérrez F, Villarroel M, María G, Estevez-Moreno LX. Welfare of horses from Mexico and the United States of America transported for slaughter in Mexico: fitness profiles for transport and pre-slaughter logistics. *Prev Vet Med.* (2020) 180:105033. doi: 10.1016/j.prevetmed.2020.105033

64. Huertas SM, van Eerdenburg F, Gil A, Piaggio J. Prevalence of carcass bruises as an indication of welfare in beef cattle and the relation to the economic impact. *Vet Med Sci.* (2015) 1:9–15. doi: 10.1002/vms3.2

65. Gallo C, Pérez S, Sanhueza C, Gasic J. Efectos del tiempo de transporte de novillos previo al faenamiento sobre el comportamiento, pérdidas de peso y algunas características de la canal. *Arch Med Vet.* (2000) 32:157–70. doi: 10.4067/S0301-732X2000000200003

66. Gallo C, Espinoza MA, Gasic J. Efectos del transporte por camión durante 36 horas con y sin período de descanso sobre el peso vivo y

algunos aspectos de calidad de carne en bovinos. *Arch Med Vet.* (2001) 3:43–53. doi: 10.4067/S0301-732X2001000100005

67. Gallo C. Using scientific evidence to inform public policy on the long distance transportation of animals in South America. *Vet Ital.* (2008) 44:113–20. Available online at: https://www.izs.it/vet_italiana/2008/44_1/113.pdf

68. Huertas SM, Gil A, Piaggio JM, van Eerdenburg FJCM. Transportation of Beef cattle to slaughterhouses and how this relates to animal welfare and carcase bruising in an extensive production system. *Anim Welfare.* (2010) 19:281–5.

69. Romero M, Sánchez V. Animal welfare during transport and its relationship with meat quality. *Rev MVZ Cordoba*. (2012) 17:2936–44. doi: 10.21897/rmvz.264

70. Gallo C, Taruman J, Larrondo C. Main factors affecting animal welfare and meat quality in lambs for slaughter in Chile. *Animals.* (2018) 8:165. doi: 10.3390/ani8100165

71. Tarazona A, Ceballos M, Broom D. Human relationships with domestic and other animals, one health, one welfare, one biology. *Animals.* (2019) 10:1–21. doi: 10.3390/ani10010043

72. Bravo V, Knowles T, Gallo C. Transport, associated handling procedures and behaviour of calves marketed through Chilean auction markets. *Animals.* (2020) 10:2170. doi: 10.3390/ani10112170

73. Sánchez M, Bravo V, Gallo C. Behavior and health indicators to assess cull cow's welfare in livestock markets. *Front Vet Sci.* (2020) 7:471. doi: 10.3389/fvets.2020.00471

74. Ocampo A, Cardozo A, Tarazona A, Ceballos M, Murgueitio E. La investigación participativa en bienestar y comportamiento animal en el trópico de América: oportunidades para nuevo conocimiento aplicado. *Rev Colomb Cs Pec.* (2011) 24:332–46. Available online at: http://www.scielo.org.co/pdf/rccp/v24n3/v24n3a14.pdf

75. Schnaider MA, Heidemann MS, Silva AHP, Taconeli CA, Molento CFM. Vocalization and other behaviors indicating pain in beef calves during the ear tagging procedure. *J Vet Behav Clin Appl Res.* (2022) 47:93–8. doi: 10.1016/j.jveb.2021.10.005

76. Ceballos MC, Sant'Anna AC, Boivin X, de Oliveira Costa F, Carvalhal M, Paranhos da Costa M. Impact of good practices of handling training on beef cattle welfare and stockpeople attitudes and behaviors. *Livest Sci.* (2018) 216:24–31. doi: 10.1016/j.livsci.2018.06.019

77. Cedeño-Palacios C, Delgado M, Duenas A, Alcivar U, Vasquez L. Compliance with good livestock practices in selected farms in Ecuador. *Rev Ci-Facultad de Ciencias Veterinarias.* (2019) 29:101–6. Availabler online at: https://produccioncientificaluz.org/index.php/cientifica/article/view/29592/30377

78. Mendes P, Siqueira H, Siqueira A, Prata L. Diagnosis of compliance with animal welfare standards in broiler slaughterhouse located in the state of Goiás. *PUBVET*. (2019) 3:5. doi: 10.31533/pubvet.v13n5a325.1-7

79. Mamani-Linares L, Gallo C. A note on the effects of pre-slaughter operations of llamas (*Lama glama*) on the concentrations of some blood constituents related to stress and carcass quality. *Arch Med Vet*. (2014) 46:463–9. doi: 10.4067/S0301-732X2014000300018

80. Smith C, Mendoza G, Gustavo C, Ghezzi M. Evaluación de las condiciones de bienestar animal de camélidos sudamericanos ingresados al camal municipal de Huancavelica, Perú. *Rev Mexicana Cs Pec.* (2019) 10:379–90. doi: 10.22319/rmcp.v10i2.4568

81. Guerrero Pincay AE, González Marcillo RL, Castro Guamàn WE, Ortiz Naveda NR, Grefa Reascos DA, Guamàn Rivera SA. Influence of litter size at birth on productive parameters in guinea pigs (*Cavia porcellus*). *Animals.* (2020) 10:2059. doi: 10.3390/ani10112059

82. Larrondo C, Orihuela A, Strappini A, Acosta G, Mota-Rojas D, Gallo C. Provision of straw and presence of undocked lambs reduce the behavioral and physiological expressions of pain and stress associated with tail docking in lambs: a preliminary study. *Anim Prod Sci.* (2020) 621:423–31. doi: 10.1071/AN20237

83. Hoetzel MJ, Sneddon JN. The role of extensionists in Santa Catarina, Brazil, in the adoption and rejection of providing pain relief to calves. *J Dairy Sci.* (2013) 96:1535–48. doi: 10.3168/jds.2012-5780

84. Larrondo C, Bustamante H, Gallo C. Sheep farmers perception of welfare and pain associated to routine husbandry practices in Chile. *Animals.* (2018) 8:225. doi: 10.3390/ani8120225

85. Larrondo C, Bustamante H, Paredes E, Gallo C. Long term hyperalgesia and traumatic neuroma formation in tail docked lambs. *Anim Welfare.* (2019) 28:443–54. doi: 10.7120/09627286.28.4.443

86. Herzberg D, Strobel P, Chihuailaf R, Ramírez-Reveco A, Müller H, Werner M, et al. Spinal reactive oxygen species and oxidative damage mediate chronic pain in lame dairy cows. *Animals.* (2019) 9:693. doi: 10.3390/ani9090693

87. Andrighetto M, Rossi J, Jardim J. Attitudes of cattle veterinarians and animal scientists to pain and painful procedures in Brazil. *Prev Vet Med.* (2020) 177:104909. doi: 10.1016/j.prevetmed.2020.104909

88. Hoetzel MJ, Yunes MC, Vandresen B, Albernaz-Goncalves R, Woodroffe RE. On the road to end pig pain: knowledge and attitudes of Brazilian citizens regarding castration. *Animals.* (2020) 10:1826. doi: 10.3390/ani10101826

89. Stampa E, Schipmann C, Hamrn U. Consumer perceptions, preferences, and behavior regarding pasture-raised livestock products: a review. *Food Qual Prefer.* (2020) 82:103872. doi: 10.1016/j.foodqual.2020.103872

90. Schnettler B, Vidal R, Silva R, Vallejos L, Sepúlveda N. Consumer perception of animal welfare and livestock production in the Araucania Region, Chile. *Chilean J Agric Res.* (2008) 68:80–93. doi: 10.4067/S0718-58392008000100008

91. Schnettler B, Vidal R, Silva R, Vallejos L, Sepúlveda N. Consumer willingness to pay for beef meat in a developing country: the effect of information regarding country of origin, price and animal handling prior to slaughter. *Food Qual Pref.* (2009) 20:156–65. doi: 10.1016/j.foodqual.2008.07.006

92. Villalobos P, Padilla C, Ponce C, Rojas A. Beef consumer preferences in Chile: importance of quality attribute differentiators on the purchase decision. *Chilean J Agric Res.* (2010) 70:85–94. doi: 10.4067/S0718-58392010000100009

93. Vargas-Bello-Pérez E, Miranda-de la Lama G, Lemos Teixeira D, Enríquez-Hidalgo D, Tadich T, Lensink J. Farm animal welfare influences on markets and consumer attitudes in Latin America: the cases of Mexico, Chile and Brazil. *J Agric Environ Ethics.* (2017) 30:697–713. doi: 10.1007/s10806-017-9695-2

94. Vargas-Bello E, Obermöller C, Faber I, Tadich T, Toro P. Knowledge and perception on animal welfare in Chilean undergraduate students with emphasis on dairy cattle. *Animals.* (2021) 11:1921. doi: 10.3390/ani11071921

95. Teixeira D, Larraín R, Hötzel M. Are views towards egg farming associated with Brazilian and Chilean egg consumers' purchasing habits? *PLoS ONE.* (2018) 13:e0203867. doi: 10.1371/journal.pone.0203867

96. Cornish A, Briley D, Wilson B, Raubenheimer D. The price of good welfare: does informing consumers about what on-package labels mean for animal welfare influence their purchase intentions? *Appetite.* (2020) 148:104577. doi: 10.1016/j.appet.2019.104577

97. Sinclair M, Zhang Y, Descovich K, Phillips CJC. Farm animal welfare science in China—a bibliometric review of Chinese literature. *Animals.* (2020) 10:540. doi: 10.3390/ani10030540

98. Huertas SM, Bobadilla PE, Alcántara I, Akkermans E, van Eerdenburg FJCM. Benefits of silvopastoral systems for keeping beef cattle. *Animals.* (2021) 11:992. doi: 10.3390/ani11040992

99. Broom DM, Galindo FA, Murgueitio E. Sustainable, efficient livestock production with high biodiversity and good welfare for animals. *Proc R Soc B Biol Sci.* (2013) 280:1771. doi: 10.1098/rspb.2013.2025

100. Tadich N, Gallo C, Brito M, Broom D. Effect of weaning and 48 h transport by road and ferry on some blood indicators of welfare in lambs. *Livestock Sci.* (2009)121:132–6. doi: 10.1016/j.livsci.2008.06.001

101. Strappini A, Frankena K, Metz J, Gallo C, Kemp B. Characteristics of bruises in carcasses of cows sourced from farms or from livestock markets. *Animal.* (2012) 6:502–9. doi: 10.1017/S1751731111001698

102. Ungerfeld R, Hötzel M, Quintans G. Changes in behaviour, milk production and bodyweight in beef cows subjected to two-step or abrupt weaning. *Anim Prod Sci.* (2015) 55:1281–8. doi: 10.1071/AN13453

103. Galindo F, de Aluja A, Cagigas R, Huerta LA, Tadich T. Application of the hands-on donkey tool for assessing the welfare of working equids at Tuliman, Mexico. *J Appl Anim Welf Sci.* (2018) 21:1. doi: 10.1080/10888705.2017.1351365

104. Hötzel M, Mota S, Ludtke C, Poletto R. Knowledge and attitudes of official inspectors at slaughterhouses in southern Brazil regarding animal welfare. *Rev Brasil Zootech Brazil J Anim Sci.* (2018) 47:65. doi: 10.1590/rbz4720170065

105. Freitas-de-Melo A, Ungerfeld R, Orihuela A, Hötzel MJ, Pérez-Clariget R. Early mother–young relationship and feeding behaviour of lambs are unaffected by low pasture allowance until the beginning of the last third of gestation in single-bearing ewes. *Anim Prod Sci.* (2017) 58: 930–6. doi: 10.1071/AN16157

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Effect of the COVID-19 pandemic and international travel ban on elephant tourist camp management in northern Thailand

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The COVID-19 pandemic has had a significant impact on the tourism industry, especially in Thailand. Starting in April 2020, the Thai government banned international travel and all elephant tourist camps closed. A wide variety of management changes were implemented because of the lack of income from tourists. This study surveyed 30 camps that cared for >400 elephants in northern Thailand to obtain information on camp, elephant, and mahout management during the COVID-19 pandemic from April 2020 to 2022 compared to the year before. The survey consisted of questionnaires that interviewed elephant camp owners, managers, veterinarians, and mahouts, and captured information on changes in camp operations, including numbers of tourists, elephants and mahouts, elephant and mahout activities, and veterinary care. Results revealed significant changes in camp structure, elephant work activities and general care. Staff layoffs led to a decrease in the ratio of mahouts to elephants from 1:1 to 1:2. Elephant activities, distance walked, and amounts of food were reduced when compared to pre-COVID-19, while chain hours were increased due to reduced activity. Overall, the COVID-19 crisis altered elephant management significantly, potentially affecting animal welfare resulting from changes in nutrition, health, exercise, and numbers of mahouts. We hope to use these data to develop better management plans and guidelines for elephant camps in Thailand so they can cope with the current and potential imminent pandemics that result in decreased tourism income. A follow-up study will measure health and welfare markers in relation to COVID-19 induced changes to determine if any camps adapted management to still meet elephant health and welfare needs, and could serve as models for responding to future pandemics.

KEYWORDS

Asian elephant, tourist camp, management, welfare, COVID-19, Thailand

Introduction

The COVID-19 outbreak that began in 2019 is notable for its high rates of infection and fatalities, and enormous economic impacts worldwide (1), including those related to tourism (2, 3). It is estimated that global production output fell by 7% when only China went into lockdown, but reached 23% at the height of the crisis when they involved other nations (4). There are strong links between the strength of the tourist industry and economic growth within a country (5). However, because tourism is dependent on numbers of visitors, it is particularly vulnerable to disruptions caused by global pandemics (6). Thus, the COVID-19 pandemic has resulted in serious and widespread negative economic impacts on the economy of countries that depend on tourism income (2, 7), especially in regions with limited resilience to pandemic losses (8).

Although there have been some positive effects of the pandemic, such as reductions in greenhouse gases and air pollution (9, 10), overall, it has had adverse effects on wildlife tourism, both for businesses and animals, *in situ* and *ex situ*, leaving it in a more vulnerable position than before COVID-19. Venues involving animals (viewing or interactions) have been particularly hard hit (11–14). Due to reduced or no income, some zoos and wildlife rescue centers closed (12), with legitimate concerns over how shortages of food and staff will impact animal welfare (11). Likewise, a reduction in wildlife tourism experiences *in situ*, such as visiting national parks, protected areas, sanctuaries, has had negative impacts on tourist hotels, travel agencies, guides, and associated local communities (15), as well as conservation efforts because tourism funds a number of projects that protect habitats and the wildlife therein (16). Some free-ranging wildlife are reportedly going hungry because a popular tourist activity is feeding; for example, sika deer in Japan (17) and rhesus monkeys in Thailand (17, 18), although in one report, free-ranging elephants in Sri Lanka returned to wild foraging after a lockdown curtailed food handouts from tourists (19).

Thailand is the epicenter of elephant tourism and visiting an elephant camp is one of the most popular activities according to the Tourist Authority of Thailand. Elephants are the national symbol of Thailand and an integral part of Thai and Buddhist culture. There are ∼3,500 captive elephants in Thailand, mostly (95%) privately owned (20, 21) and used primarily for tourism; thus they are also important to national economics. Most captive elephants in Thailand are in the north and northeast part of the country (∼60%), primarily in Chiang Mai province (22). A recent survey of 33 elephant camps differing in size and years of operation in the region (23) found tourist activities varied and included hands-off opportunities like observation from afar, to feeding, bathing, and walking alongside, and to more interactive activities like riding with a saddle or bareback, and elephant shows. The question has always been – how do these tourist activities affect elephant health and welfare? Thus, a further evaluation of 122 elephants from 15 elephant camps using physical assessments of body condition, foot, and wound scores found that high energy foods (banana and sugar cane) were associated with obesity and alterations in total cholesterol (TC), low density lipoproteins (LDL), high density lipoproteins (HDL), triglycerides (TG), insulin, glucose, fructosamine and the ratio of glucose to insulin, while fecal glucocorticoid metabolite (fGCM) concentrations were lower in riding elephants, perhaps related to more exercise and better body condition (24, 25). However, poor foot scores were associated with longer work hours and walking distances and being on concrete, while skin wounds were related to improper restrain equipment used by mahouts (e.g., ankus or bullhook, chains) (26). Thus, while some tourist activities may benefit elephant health (24), others can contribute to poor welfare through long work hours, misuse of the ankus, stress associated with being too close to tourists, and harsh training to allow hands-on interactions (26, 27).

When the COVID-19 pandemic hit, the tourism landscape changed drastically. Upon recognition of the virus in March 2020, the Thai government banned all international travel (28), severely reducing foreign tourism and associated income. Consequently, tourist camps closed in Thailand, leading to further concerns over welfare of the elephants and mahouts. Therefore, the goal of this study was to document how elephant management changed a result of the international travel ban due to COVID-19. Surveys were conducted throughout the first 2 years of the country-wide lockdown, with data compared to before COVID-19 [(24, 25, 27), this study]. This information will then be used in subsequent multivariable studies to assess how management changes affected physiological function. It also will be used to devise plans for dealing with future pandemic-induced losses of income and identify areas that camps need to improve upon to adapt to inevitable future pandemics.

Materials and methods

Human ethical consent

This study was approved by the Faculty of Veterinary Medicine, Chiang Mai University Research Ethics Committee (HS1/2564).

Animal ethical consent

This study was approved by the Institutional Animal Care and Use Committee, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand (FVM-ACUC, permit number S4/2564).

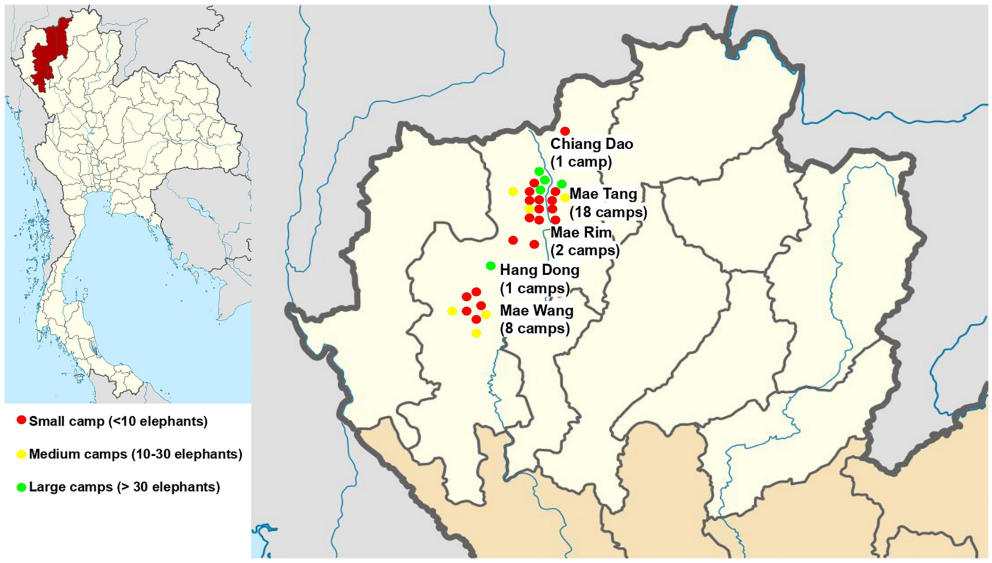

FIGURE 1
Distribution of elephant camps in this study. Colored dots represent the size of elephant camps based on numbers of elephants.

Data collection

Data collection was carried out from April 2020 to April 2022. A total of 30 camps in five districts in Chiang Mai province were surveyed: Chiang Dao (one camp), Mae Tang (18 camps), Mae Rim (two camps), Hang Dong (one camp) and Mae Wang (eight camps) (Figure 1). These camps housed 495 elephants: 119 males (18.37 ± 1.67, range 3 months to 57 years of age) and 376 females (27.54 ± 0.94, range 8 months to 70 years of age), at the beginning of the study. Of these camps, 56% (n = 17) were considered small (<10 elephants), 27% (n = 8) were medium (10–30 elephants), and 17% (n = 5) were large (>30 elephants). Camps had been in operation for 0-5 (40%, n = 12), 6–15 (30%, n = 9) or >16 (23%, n = 7) years as of April 2020.The study consisted of questionnaire interviews with camp owners, managers, and/or camp veterinarians, and direct observations at elephant camps (Supplementary Table 1). Interviewers and observers were veterinarians experienced in working with elephants from the Veterinary Faculty at Chiang Mai University. The questionnaire consisted of questions that took approximately 60–90 min to complete: (1) camp management including sanitation, years of operation, elephant numbers, staff numbers, location, number of tourists, rest areas; (2) elephant management including tourist activities, chaining, restraint, access to drinking water, and musth management and nutrition; (3) mahout responsibilities, salaries and attitudes; and (4) health care consisting of sanitation practices, deworming program, veterinary care, and external sponsorship and funding support. Questions about camp management before COVID-19 were included in the first survey to capture data on operations in 2019 (Supplementary Table 1). Additional information on camp management and elephant activities before the COVID-19 pandemic also was available from Bansiddhi et al. (23). Follow-up surveys were then conducted every 4 months through April 2022 for a total of 2 years during the lockdown and international tourism ban (Figure 2).

Statistical analysis

Descriptive statistics are reported as a percentage and the mean ± standard error of the mean (SEM). Statistical analyses were conducted using R program (version 3.4.0). Repeated survey data were analyzed using Generalized Estimating Equations (GEE) to determine how camp management variables changed over time (T00–T06). Differences in mean camp management variables (elephant numbers, staff numbers, mahout number, number of visitors, chain hours, frequency of access to drinking water, amount of roughage food, amount of high calories treats, and mahout salary) between times during COVID-19 were analyzed using by Dunnett's test using a P-value correction. Statistical significance was set at $P < 0.05$.

Results

The timeline for associated events before the COVID-19 pandemic (T00), and across the six survey periods (T01–T06)

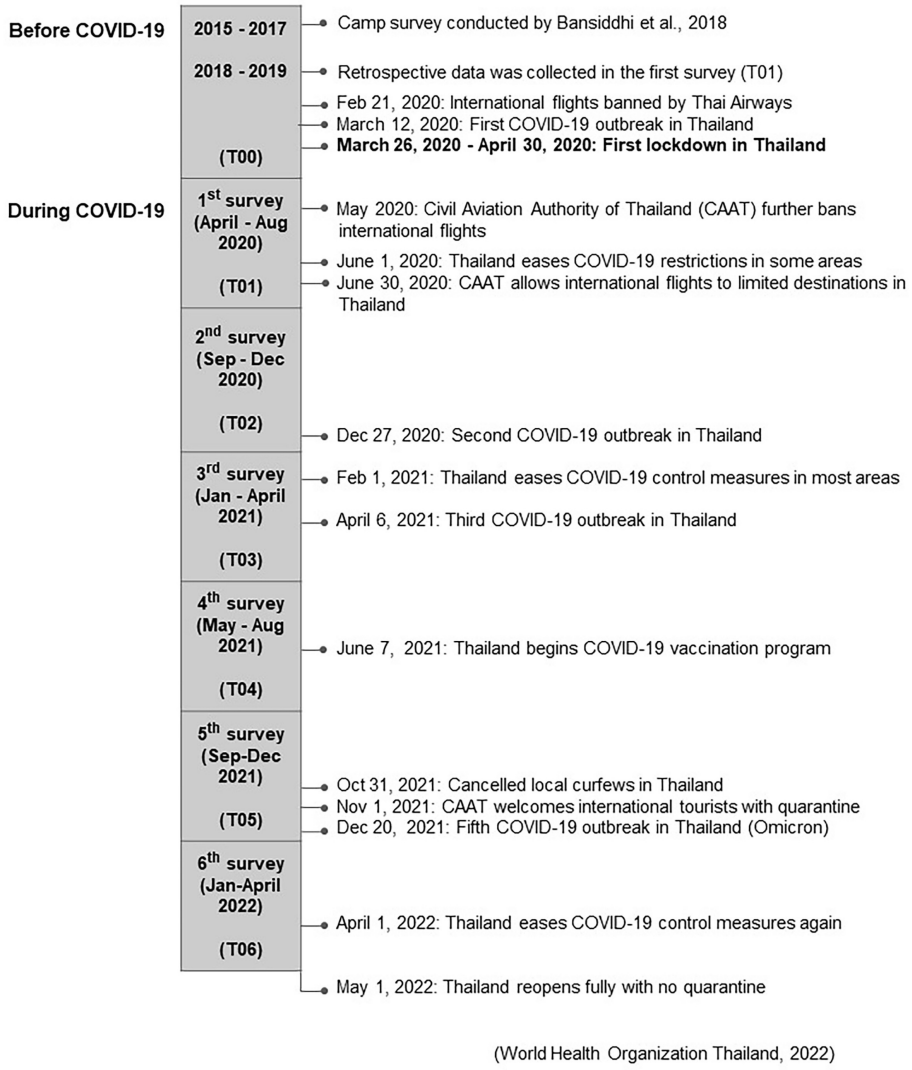

FIGURE 2
Associated events before the COVID-19 pandemic (T00), and across the six survey periods (T01-T06) during the study.

during the study is shown in Figure 2. Thailand did not fully open to international travelers with no restrictions until 1 month after the last survey.

Visitor, elephant, mahout, and staff numbers

The international travel ban initiated by the Thai government in April 2020 was followed by an immediate reduction in the number of visitors in T01 (Table 1, Supplementary Figure 2A), with no tourists visiting 60% of the camps ($n = 17$) and <1% of original tourist numbers in the rest, all of those being local Thais only. Tourist numbers remained low even as some restrictions were lifted in mid-2020, when international travel was allowed, but with limitations (quarantine for 14 days and only in some locations) (Figure 2). In the last two surveys, visitor numbers had begun to increase again, but were still only 7% of pre-COVID numbers (Table 1, Supplementary Figure 2A).

Elephant numbers at each camp were decreased by about 11% soon after camps closed to 39% at the end of the survey period (Table 1, Supplementary Figure 2B). At some camps, mahouts returned elephants to their home village (56.7%, $n = 17$), while some were sold to other camps (30%, $n = 9$). Three mahouts (10%) took elephants to log in Surin province, while two owners allowed elephants to stay at a temple (6.7%, $n = 2$) (Table 1, Supplementary Figure 2C). There was a 45% decrease

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TABLE 1 Summary of parameters (mean ± SEM, range) related to management of elephants in tourist camps in Chiang Mai province, Thailand, in each period from surveys conducted over 2 years during the COVID-19 pandemic compared to the year pre-COVID-19.

Parameters	T00 before COVID-19[1]	Time periods during COVID-19					
		T01 (April 2020–August 2020)	T02 (September 2020–December 2020)	T03 (January 2021–April 2021)	T04 (May 2021–August 2021)	T05 (September 2021–December 2021)	T06 (January 2022–April 2022)
Visitors/day	99.82 ± 30.00[a]	1.74 ± 0.67[b]	2.18 ± 0.87[b]	2.18 ± 0.87[b]	1.82 ± 0.87[b]	4.21 ± 1.14[b]	7.39 ± 1.12[b]
	8–600	0–15	0–20	0–20	0–20	0–30	0–30
Number of elephants	16.50 ± 3.62[a]	14.66 ± 3.45[b]	14.34 ± 3.41[b]	13.38 ± 3.28[b]	12.97 ± 3.23[b]	12.63 ± 3.13[b]	11.83 ± 2.86[b]
	2–69	1–67	0–65	0–63	0–59	0–55	0–55
Number of mahouts	16.37 ± 3.71[a]	9.00 ± 1.88[b]	8.89 ± 2.07[b]	8.07 ± 1.87[b]	8.00 ± 1.88[b]	7.50 ± 1.67[b]	6.79 ± 1.40[b]
	2–66	0–40	1–40	1–39	1–39	1–32	1–30
Mahout/ elephant ratio	0.99[a]	0.64[b]	0.58[b]	0.56[b]	0.56[b]	0.55[b]	0.54[b]
	1:1	1:2	1:2	1:2	1:2	1:2	1:2
Number of Staff	30.5 ± 7.97[a]	14.90 ± 3.53[b]	14.97 ± 3.52[b]	11.55 ± 2.59[b]	11.55 ±2.59[b]	11.17 ± 2.38[b]	9.59 ± 2.16[b]
	4–209	3–80	3–80	2–60	2–60	2–50	2–45
Walk distance (km/day)	4.12 ± 0.70[a]	1.28 ± 0.15[b]	1.04 ± 0.16[b]	0.76 ± 0.11[b]	0.85 ± 0.10[b]	1.29 ± 0.12[a]	1.54 ± 0.15[a]
	0.6–20	0.3–4	0.5–3	0.3–3	0.3–3	0.5–3	0.5–3
Access to water/day[2]	3.33 ± 0.12[a]	2.90 ± 0.07[b]	2.00 ± 0.12[b]	1.27 ± 0.10[b]	1.23 ± 0.09[b]	1.23 ± 0.09[b]	1.23 ± 0.09[b]
	2–4	1–3	1–3	1–3	1–3	1–3	1–3
Chain time (hours)[2]	15.85 ± 0.42[a]	18.97 ± 0.63[b]	21.47 ± 1.24[b]	23.96 ± 1.52[b]	25.75 ± 1.72[b]	23.75 ± 1.53[b]	21.16 ± 1.06[b]
	0–19	0–24	0–48	0–48	0–48	0–48	0–48
Chain length (m)	3.85 ± 0.47[a]	5.47 ± 0.78[b]	5.45 ± 0.79[b]	5.31 ± 0.81[b]	5.35 ± 0.80[b]	5.09 ± 0.79[b]	5.09 ± 0.79[b]
	0–12	0–15	0–15	0–15	0–15	0–15	0–15
Roughage (kg/day)	213.45 ± 14.07[a]	208.3 ± 14.10[b]	173.45 ± 10.32[b]	164.29 ± 8.02[b]	148.21 ± 6.28[b]	147.86 ± 6.01[b]	152.50 ± 6.62[b]
	100–400	100–400	80–300	90–250	90–200	90–200	90–250
Supplements (kg/day)	26.0 ± 1.82[a]	19.0 ± 1.32[b]	10.5 ± 0.94[b]	6.5 ± 0.61[b]	6.3 ± 0.55[b]	6.3 ± 0.55[b]	9.6 ± 0.51[b]
	10–50	10–30	2–25	1–15	5–15	5–15	5–15

[1]Based on interview questions included in T01 survey about conditions in 2019. [2]Some elephants were chained for more than 24 hours at a time, so these data represent contiguous hours in any one time period. [a,b]Different letters in the same row indicate significant statistical differences compared each time period to before COVID (T00) when subjected to Dunnett's Multiple Comparison ($P < 0.001$).

in mahout numbers almost immediately that then stabilized through 2021 (Table 1, Supplementary Figure 3C) dropping to a low of 59% of pre-COVID numbers in T06. Overall, the decrease in numbers of elephants was less than the reduction in numbers of mahouts so the overall ratio of mahouts to elephants dropped from around 1:1 at T00 to 1:2 throughout T01–T06 (Table 1). A 50% reduction in other staff, including gardeners, drivers, cleaners, cooks, and guides also was observed across facilities shortly after the lockdown in T01 (Table 1, Supplementary Figure 2D), with the lowest percentage (29%) observed at the end of the study.

Work activities

Pre-COVID-19 information collected as part of the initial T01 survey (designated T00) found the main tourist activities were no-riding and bathing (27% of camps) followed by feeding (37%), and then bareback (12%) or saddle (10%) riding, and shows (8%) (Figure 3, Supplementary Figure 1). Additional activities not described in prior studies included coffee café with elephants (5%), where a group of tourists interact with elephants by feeding bananas or sugar cane and/or observation from the coffee bar, and camping with elephants (1%), where tourists stay overnight in a tent with feeding and observation opportunities. With no tourists, elephant activities in the majority of camps ceased and so there was little if any exercise in the form of riding, foraging, or other work (Figure 3). At the beginning of study (T00), elephants walked on average over 4 km/day part of tourist activities, with some walking up to 20 km/day (Table 1, Figure 4A). After the lockdown, daily walking distances at most camps (70%, $n = 21$) were reduced, with a low in T03 (< 0.8 km/day). As shown in Figure 4A, no camps walked elephants less than 0.6 km/day before COVID-19, while no elephants were

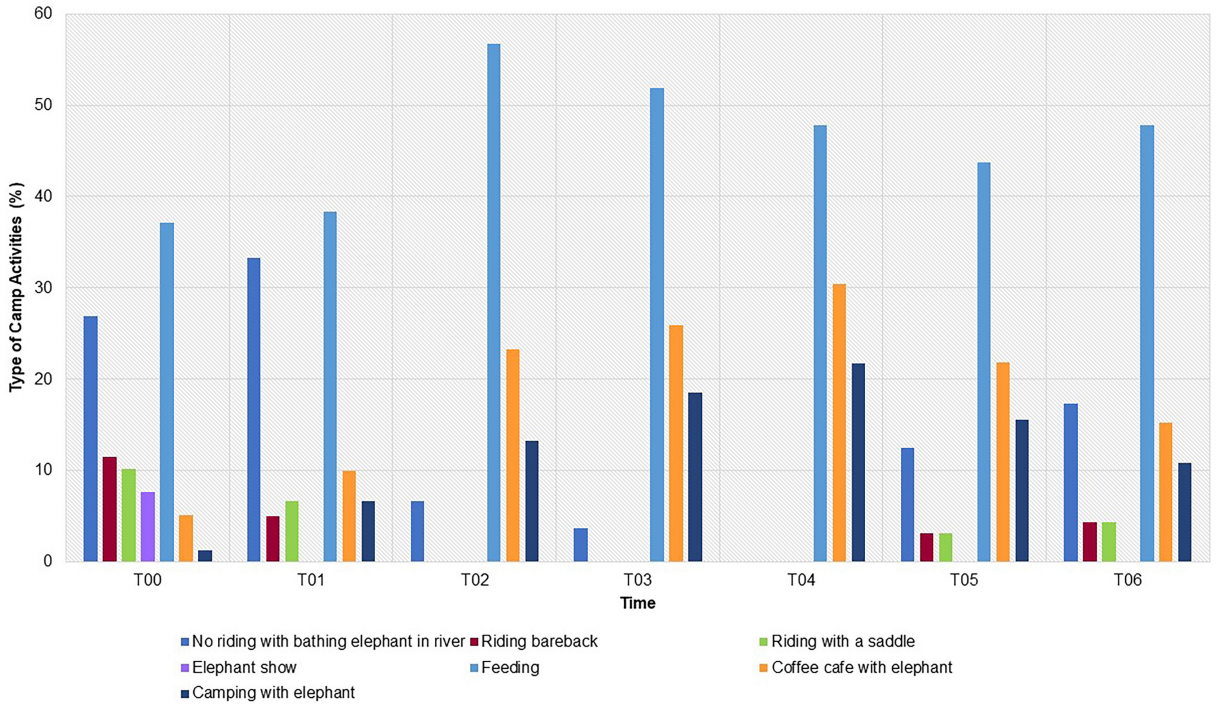

FIGURE 3
Changes in tourist activities at elephant camps in northern Thailand before (T00) and through six survey periods [T01 (April–August 2020), T02 (September–December, 2020), T3 (January–April, 2021), T04 (May-August 2021), T05 (September–December, 2021) and T06 (January–April, 2022)] during the COVID-19 pandemic. Data represent the percentage of camps engaged in each activity across time periods.

exercised more than 4 km/day after camps closed in T01. Riding activities declined to less than 10% in T01 and were halted altogether through T05, when a small number of local tourists (<5% of camps) returned for these activities (Figure 3). At the end of the study, the percentage of camps providing at least some walking opportunities was 47% (n = 14) (Figure 3). By contrast, 46% of camps (n = 19) continued to allow local tourists to feed supplements purchased for elephants, like bananas and sugar cane, throughout the study period. Other activities that appealed to local Thai people increased, such as coffee café and elephant camping, which made up a greater percentage of activities involving elephants as the pandemic progressed, in addition to feeding (Figure 3).

Before COVID-19, mahouts bathed elephants in a river (53% of camps), often with tourists, or by spraying with a hose (66%) at a frequency of two (23%) to four (30%) times per day Table 1, Figure 3). When camps closed, bathing frequency was reduced to 1–3 times per day, but over time, fewer camps were doing it. By T03, bathing times were less than half those in T01, and many camps (80%) stopped bathing altogether. Whereas before COVID-19, one mahout would bathe one elephant, at the end of the study a mahout might bathe a group of elephants, and at a decreased frequency (Table 1).

Chaining, housing, rest areas

With the reduction in work activities, there was an increase in chaining time at 77% of the camps (n = 23) (Table 1, Figure 4B). Chaining time already averaged 16 hours/day before the lockdown, although there was considerable variability across camps, ranging from 0 to 19 h in T00 and 0–48 h in T06 (Table 1, Figure 4B). By T02, some camps (7%, n = 2) started chaining elephants for 48 straight hours. Only four camps (13%) allowed elephants to roam freely without chaining, and that stayed constant throughout the study (Table 1, Figure 4B). No camps chained elephants for >21 h before COVID-19, but after T03, most did (Table 1, Figure 4B).

At the beginning of the study, chain lengths at most camps were 2.1–3.0 meters (T00), with only 10% using chains >5 m (Table 1, Figure 4C). After the lockdown (T01), 23–27% of camps increased the length of the chains used. By contrast, chain lengths were shortened at three camps because of limited and more restricted space (Table 1, Figure 4C). During COVID-19, most camps (67%, n = 20) chained elephants under a covered shed, while some (33%, n = 10) kept animals in sheds and/or woodlands. In three camps, elephants were allowed to roam free in neighboring forests while being restrained by heavy chains, while at two, they were allowed to roam freely around the camp.

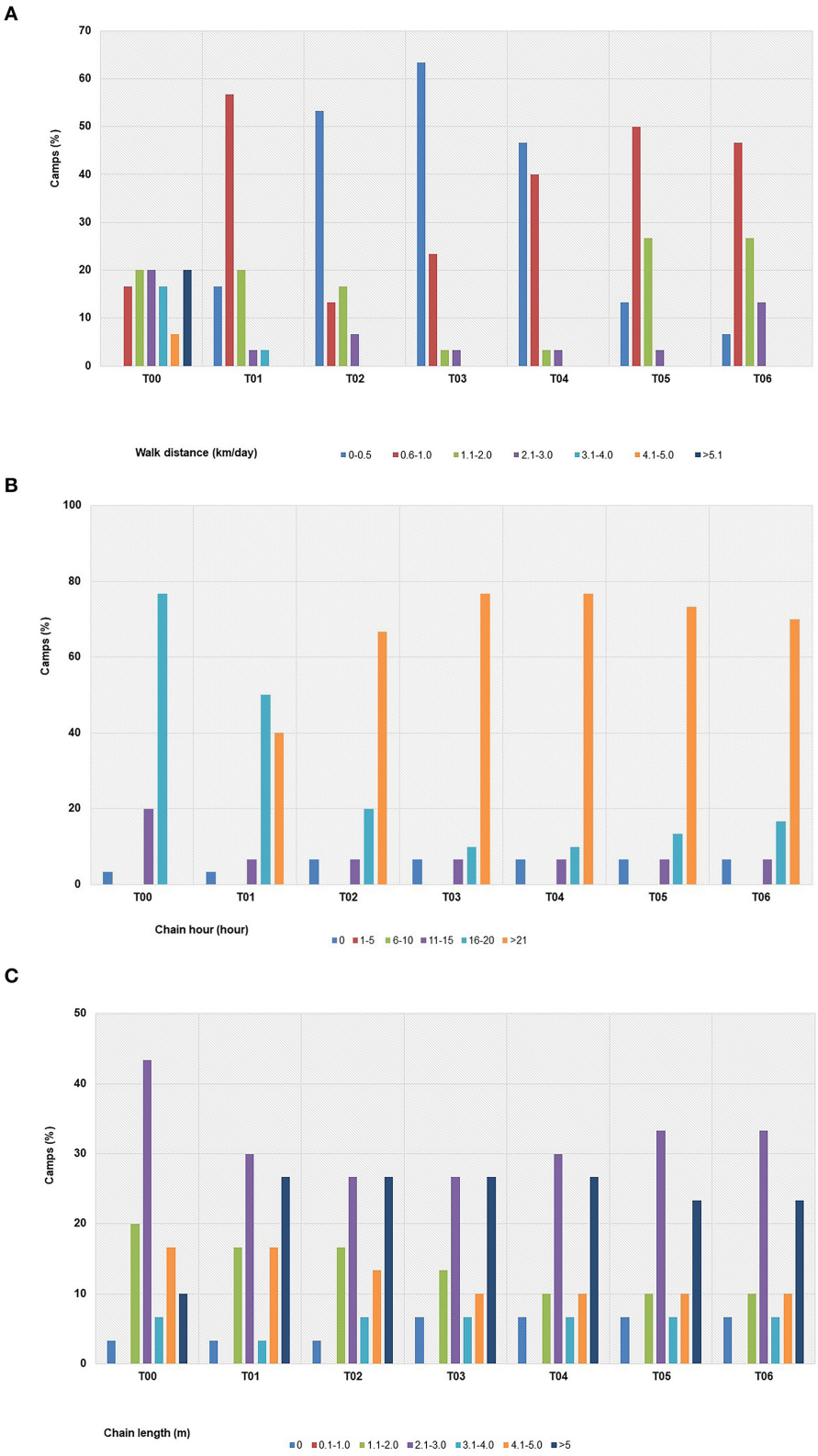

FIGURE 4
Changing trends of elephant exercise **(A)** walking distance, **(B)** chain hours, and **(C)** chain length at individual elephant tourist camps in northern Thailand before (T00) and through six survey periods [T01 (April–August, 2020), T02 (September–December, 2020), T3 (January–April, 2021), T04 (May–August, 2021), T05 (September–December, 2021) and T06 (January–April, 2022)] during the COVID-19 pandemic.

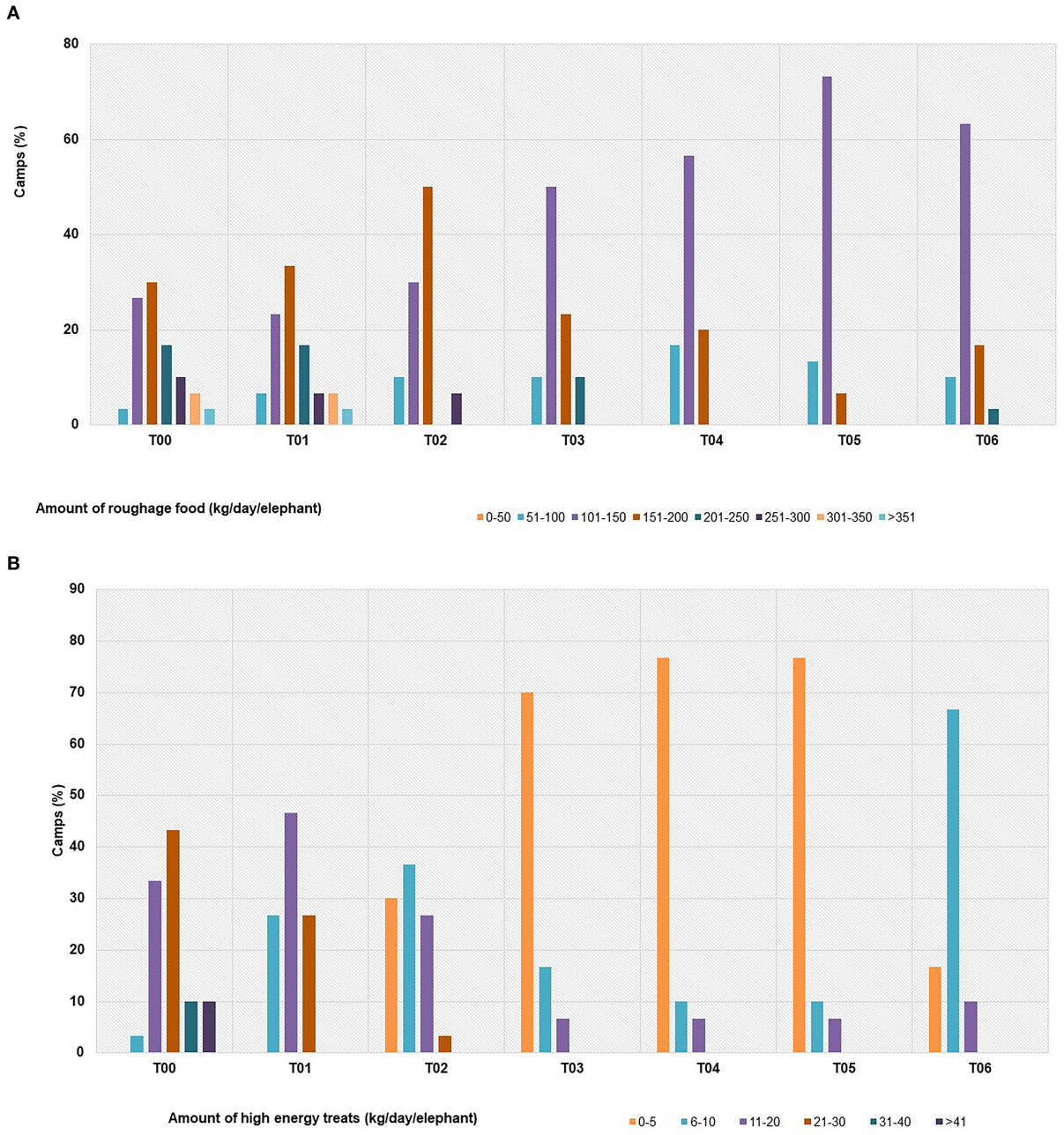

FIGURE 5
Changing trends of food provided **(A)** roughage **(B)** supplement at individual elephant tourist camps in northern Thailand before (T00) and through six survey periods [T01 (April–August, 2020), T02 (September–December, 2020), T3 (January-April, 2021), T04 (May-August 2021), T05 (September–December, 2021) and T06 (January-April, 2022)] during the COVID-19 pandemic. Types of high energy treats are described in Supplementary Figure 3B.

Nutrition

The types of roughage offered did not change significantly during COVID-19 (Supplementary Figure 3A), although the amounts fed were reduced over time, averaging only ∼70–80% of those in T00 (Figure 5A and Table 1). The vast majority of camps fed napier grass, which continued throughout the study (Table 1, Supplementary Figure 3A); however, the number of camps feeding cornstalks declined from 67% in T00 to 47% from T01 onwards (Supplementary Figure 3A). In T04,

over half of the camps tried feeding straw, but that was discontinued by the next survey (Supplementary Figure 3A). Elephants were fed a variety of supplements before COVID-19, most commonly bananas, sugar cane and tamarind (Table 1, Supplementary Figure 3B). These items continued to be offered through 2020, although fewer camps did so; 86% of camps fed sugar cane in T00 but only 40% did in T01, while tamarind went from 100% to less than 3% in just a few months (Figure 5B). Overall, the amount of supplemental, higher calorie food was reduced by 57% across camps (Figure 5B and Table 1), going from feeding 10–50 kg/day in T00 to 5–15 kg/day in T06. Beginning in T01 some supplements like bananas, sugar cane and other seasonal fruits like pumpkin, watermelon, cantaloupe, melon, and mango were donated by local Thai people.

Health care

Before COVID-19, four camps had their own full-time elephant veterinarian on site, while other camps were visited twice a year by veterinarians from the National Elephant Institute (NEI) ($n = 5$), the Center of Elephant and Wildlife Health, Chiang Mai University (CMU) Animal Hospital ($n = 9$), the Department of Livestock Development (DLD), National Institute of Elephant Research and Health Service ($n = 1$), or the Thai Elephant Alliance Association (TEAA) ($n = 3$) that conducted routine health checks and provided deworming services. After the lockdown, only three camp veterinarians remained, and all at a reduced salary (20–30% of T00). Numbers of veterinarians working for the TECC, CMU, DLD and TEAA remained the same and they continued to visit camps for routine care, but took on additional tasks, such as foot care, gastrointestinal tract (GI) treatment, wound care and other health problems because of the reduction in mahouts and elephant exercise activity.

Mahout management and mahout attitudes

Mahouts continued to care for all aspects of the elephants' daily lives, including walking, providing food and water, cleaning enclosures, and bathing (Supplementary Figures 1, 4), although amounts of time devoted to these activities often were reduced. A total of 87% ($n = 214$) of surveyed mahouts answered questions about attitudes toward management changes during COVID-19 (Table 2, Supplementary Figure 4). Mahout salaries were reportedly decreased by 60% during the pandemic, as were self-reported feelings of stress and sadness (Table 2), although by T06, those feelings had decreased somewhat. By contrast, fear of layoffs was reported by only by a third of mahouts soon after camps closed, but increased as the pandemic progressed to over 90% in T04–T05 (Supplementary Figure 2C). By the last survey, the percentage was still close to two-thirds. Some mahouts reported getting second jobs, such as a gardener or construction worker, depending on the camp.

Discussion

This study presents survey results on the effects of the COVID-19 pandemic and international travel ban on elephant tourist camp management in northern Thailand. The study population represented 61% of the total elephant numbers in the Chiang Mai region (14% overall in Thailand). Compared to pre-COVID-19, data revealed major changes in camp and elephant management occurred as a result of a loss in tourism income. Reductions in exercise opportunities, increases in chaining time, changes in diets, and loss of mahouts all were observed and fully expected to have significant impacts on animal wellbeing. In addition to surveys, biological samples and health data also were collected for future studies to measure physiological responses (i.e., body condition, stress, metabolic, liver, muscle function, and behavior), data that will be important to understanding how changes in diet, health care, and exercise affected aspects of individual elephant welfare.

Visitor, elephant, mahout, staff numbers

In the present study, elephant numbers declined by more than 30% over time as mahouts returned to villages or elephants were sold, whereas as staff was reduced by 50% or more as the pandemic progressed. In Nepal, the captive elephant population also decreased by 18.5% during COVID-19 since an earlier report in 2012, in part related to illegal selling of privately owned elephants to Indian entrepreneurs (29). To our knowledge, there are no other studies documenting the effect of the COVID-19 pandemic on the management of elephants used primarily for tourism. However, it can be interfered that changes in camp management, including reducing the mahout to elephant ratio, will have significant effects on health and welfare, and cause stress in elephants forced to adapt to new environments (30).

Work activities

Before COVID-19, elephants generally worked from 8.00–10.00 to 14.00–15.00 h depending on seasonal tourist activities, and were chained primarily during non-tourist hours (31). The types of elephant tourist activities identified in T00 (before COVID-19) were similar to those reported earlier and included

TABLE 2 Mean (± SEM) and percentage of answers on the mahout surveys ($n = 214$) conducted over 2 years during the COVID-19 pandemic.

Parameters	Time periods during COVID-19					
	T01 (April 2020-August 2020)	T02 (September 2020-December 2020)	T03 (January 2021-April 2021)	T04 (May 2021-August 2021)	T05 (September 2021-December 2021)	T06 (January 2022-April 2022)
Mahout salaries (Baht Thai)[1]	4,900 ± 381[a]	4,736.84 ± 363[b]	4,070.95 ± 363[b]	4,070.95 ± 363[b]	4,070.95 ± 363[b]	4,070.95 ± 363[b]
	3,000–9,000	3,000–9,000	3,000–9,000	3,000-9,000	3,000–9,000	3,000–9,000
Mahout attitudes						
Feel stressed (%)	87.36[a]	81.9[b]	86.16[b]	83.33[b]	76.19[b]	63.16[b]
Feel sad (%)	78.78[a]	59.47[b]	67.24[b]	46.30[b]	38.10[b]	31.58[b]
Worried about layoffs (%)	33.62[a]	69.44[b]	78.37[b]	92.59[b]	90.48[b]	52.63[b]

[1]Mahout salaries before COVID-19 averaged 10,048 ± 754 Baht Thai. [a,b]Different superscript across rows indicate significant statistical differences compared each time period to T01 when subjected to Dunnett's Multiple Comparison ($P < 0.001$).

riding with a saddle, riding bareback, no-riding, bathing, and shows (23). Before the pandemic, walking distances averaged 4 km/day, with some elephants walking up to 20 km/day during trekking. Those distances were comparable to earlier findings of approximately 5–10 km/day in North American (32), Melbourne (33) and Dublin (34) zoos, tourist camps in Thailand (23), forest camps in India (35), and estimates for wild elephants (36–38). These were drastically reduced within months of the lockdown and remained low throughout the study period. However, there were four camps that made an effort to take elephants for walks, albeit at a reduced frequency. This is concerning because a previous study in North American zoos showed elephants that walked 14 h or more per week were at a reduced risk of being obese (39), a problem identified in Thailand that was ameliorated by exercise (e.g., riding) (24, 25, 40). However, it is important to point out that although riding and other activities can be good for general body condition and metabolic health (24, 25), the amounts and types of work, and training needed for elephants to participate in interactive tourist activities can have numerous negative consequences (27).

In northern Thailand, the process of Phajaan was originally designed to break an elephant's spirit so it could be handled more easily, and generally included restraining in a small enclosure with chains and harnesses to limit movement, hitting with an ankus, and then rewarding with bananas over a period of 5–10 days (31, 41). Today, Phajaan is mostly ceremonial with blessings conducted to prevent bad spirits from harming the calf. Some camps train their own baby elephants, while others send them to the National Elephant Institute (NEI), where more positive methods are now being used and based on training provided by western experts (41). In the livestock industry, Grandin (42) noted that working with large animals carries some inherent risks, and that training animals to cooperate with handling techniques can lessen anxiety and accidents. While more camps report using positive training techniques today, most elephants are still controlled with an ankus (i.e., bullhook; 85% of camps) (23), which if used improperly can injure elephants (31). For example, 27% of elephants controlled by an ankus had associated wounds, and higher wound scores were associated with higher fGCM concentrations (26, 27). Ill-fitting saddles or inadequate or inappropriate padding material also can cause lesions (43), and although not properly studied, the shape of the backbone is believed to play a role, with higher ridgelines being more prone to saddle injuries. Following this study, improvements in saddles and padding were made (43), resulting in fewer lesions (5%) in a subsequent survey (26), while another study showed carrying loads up to 15% of the elephants' body weight did not alter gait dynamics (44).

A small percentage of camps (~8%) put on elephant shows, which have their own welfare concerns. Hernias, arthritis, lameness, and joint issues may be caused by repeated abnormal positions during performances, as has been shown in circus elephants (45). These shows were curtailed soon after the lockdown in T01. Finally, it is not always clear how or if camps are addressing the mental health needs of elephants, particularly in relation to socialization (21, 27). However, one positive sign from a 2018 survey is that newer camps appear to be providing more opportunities for elephants to be together, to socialize and play, especially during bath time (23). Positive social connections between animals, even those that are not related, can operate as a calming force against difficult situations and improve general health and wellness (46–48). However, any progress in this area was curtailed during the COVID-19 lockdown, when most elephants were chained for prolonged periods of time with no ability to socially interact.

Chaining, housing, rest areas

Chaining is a way to restrict movement of elephants at facilities with limited space or no other means of containing

them. The vast majority of camps in northern Thailand use chains to control elephants, especially at night; only a few have enclosures to allow elephants untethered movements (23). Even before the pandemic, elephants in this study were chained on average nearly 16 h/day. That increased to up to 48 contiguous hours at some camps. Chaining for extended amounts of time to restrict movement can cause problems with joints and feet (49, 50) and be a source of psychological stress. In a recent survey of 283 elephants at 20 elephant camps in Chiang Mai province conducted the lockdown, 57% exhibited stereotypic behavior (51), an indicator of poor welfare. Swaying was the most common, followed by weaving and pacing, and was more common in younger elephants. Previous research has demonstrated a strong positive association between chaining and the degree of stereotypic behavior compared to elephants kept in an enclosed space that allows some free movement (52, 53). The Food and Agriculture Organization of the United Nations published a Elephant Care Manual for Mahouts and Camp Managers a decade ago that states that chains to confine adult elephants in Asia should be 20–30 m in length (54), which is rarely adhered to in Thailand; chains typically average 3 m during the day and 6 m at night (23). In southern India, a higher prevalence of stereotypies were observed in elephants chained for 20 and 18 h/day in Hindu temples (49%) and private camps (25%), respectively, compared to those chained by the Forest Department for only 6 h/day (7%) (55). In western zoos, chaining is acceptable during medical treatments or other short-term interventions, but not for prolonged restraint. In the current study, average chain length was only 2–3 m at beginning of the study, but was increased to more than 5 m after T05 potentially to help mitigate the reduction in activity levels, but also because the density of elephants under a shelter was also lower. Western zoos require elephants have access to both indoor and outdoor spaces (56) and for the most part, elephants in Thailand were kept in covered sheds or forest canopies (23).

Nutrition

Few camps in Thailand are located in forested areas that allow elephants to forage naturally, and even those that are still have to supplement because of degraded habitats, especially during the dry season (57). In general, elephants consume about 5% of their body weight on a wet weight basis, depending on sex and age; thus, an elephant cow needs 150–175 kg/day while bulls require 200–275 kg/day (58). Before COVID-19, elephants were fed roughage before morning work activities at 6.00–8.00 h and again at 17.00–21.00 h in the evening (23), and that was still the case during the pandemic. However, while the average amount of roughage offered was similar to other studies in T00, it was reduced from 200 kg/day to 150 kg/day during the pandemic. At most camps, tourists often pay to feed elephants a number of supplementary foods, such as banana and sugar cane and other seasonal fruits, which often reach 30 kg/day during the high tourist season (40). That was similar to the ∼26 kg/day amount fed pre-COVID-19, but was reduced significantly to a low of 6.3 kg/day in T04, and provided mostly by local Thai tourists. Although not quantified, a reduction in foraging at some camps was an indirect consequence of the lack of tourists, and also reduced numbers of mahouts, taking them for walks in the forest. One question to be addressed in follow up studies is how changes in diet affected body condition and metabolic activity, and whether more limited feeding of high calorie treats might benefit elephant health and reduce the incidence of obesity, or would those improvements be offset by concomitant reductions in physical activity.

Health care

Although the number of elephant veterinarians did not change significantly during the COVID-19 pandemic (only one camp veterinarian was let go), salaries were reduced and attitudes were negatively affected. There also was an increase in reported elephant health problems during the shutdown between 2019 and 2022 (59), presumably due to reduced care with fewer mahouts being available to do daily health checks. Likewise, more incidences of colic could have been related to reductions in exercise and associated impaired GI movement, in addition to poorer quality roughage.

As the pandemic progressed and camp incomes were reduced, veterinarians and veterinary assistants were increasingly supported by outside organizations, including Asian Elephant Support, Southern Thailand Elephant Foundation through the Thai Elephant Alliance, the Thai Elephant Federation, GTAEF Helping Elephants Foundation, and the Elephant Care International Healthcare and Welfare Lifeline Fund. In addition, there was some government assistance from the Tourism Authority of Thailand (TAT) to help elephant communities, and low-interest loans were provided by the Ministry of Finance for elephant camp operators.

Mahout management and attitude

Many years of research in the livestock industry have highlighted the significance of good human-animal relationships (HARs) on animal welfare and productivity, leading to recommendations for stockpersons to undergo cognitive-behavioral training as well as the inclusion of HAR assessments in on-farm welfare audits (60–62). Mahouts play an important role in the life of elephants, both positive and negative. They can engender fear as in punishment for misbehavior or gradually strengthen and foster compassionate relationships

(31). Mahouts and elephants often develop special bonds that are rarely found in other human-animal interactions, and can have positive impacts on health and welfare (63, 64). Ultimately, the wellbeing of elephants is inextricably tied to the experience and compassion of mahouts, which unfortunately appears to be dwindling across Asia (63, 65). Strong ties between mahouts and elephants also can predict levels of cooperation. When elephants were asked to cross a novel surface (low bridge), those that had worked with their handler for over a year were more willing to cross it than those with a shorter relationship (66). Likewise, elephants responded more, and faster, in behavioral tasks in response to mahouts they had known longer (63). In zoo elephants, positive keeper attitudes were related to lower mean serum cortisol concentrations as a measure of stress, while keeper work satisfaction was predicted by the strength of keeper-elephant connections (67).

Given mahout welfare is a critical component of elephant welfare, the mental health and physical fitness of mahouts is so important (68). The COVID-19 pandemic dramatically affected mahouts, not just in terms of salary but overall attitudes and quality of life (35, 68). As mahout salaries were reduced, feelings of stress and sadness increased. In particular was an increased concern over layoffs as the pandemic proceeded. Thus, it was clear that plans to deal with future pandemics must include ways to support mahouts as the centerpiece of elephant care and welfare.

Conclusion

This study found the COVID-19 pandemic had direct and significant effects on elephant camp management as a result of a loss in tourist income. Reductions in exercise opportunities and food provided, increases in chaining time, and fewer mahouts were observed, which could have significant impacts on elephant welfare. The next step will be to correlate measures of body condition, fGCM concentrations, metabolic and muscle function biomarkers, lipid panels, and behavior to determine how these management changes affected the health and welfare of specific elephants. It will also be key to identify any camps that adapted management in a way that still met elephant health and welfare needs, and which could serve as models for responding to future pandemics.

There were several notable findings from the responses to this pandemic. One was that most elephants in Thailand are located in areas with limited access to natural habitats for foraging. Before the pandemic, this problem was mitigated by large numbers of tourists providing an income to camps to purchase roughage, and by buying treats to feed elephants directly. In addition, at many camps, elephant care was based on daily tourist activities (feeding, walking, trekking, etc.) rather than allowing elephants to roam free to forage and socialize as a means of exercise. Therefore, when guests were not around, elephants were simply chained. To plan for future pandemics, while it is not possible for all camps in Chiang Mai at the present time, it is strongly recommended that they be established near forests to provide adequate space for elephants to roam and forage regardless of whether tourists are around or not. However, resistance by government or community agencies to allowing elephants access to forested areas for fear habitat would be destroyed in the long-term, is an impediment. Some camps have planted grass fields and grow their own food, a solution that could be expanded to other facilities. Those actions could reduce the food budget, while foraging would serve as natural enrichment. Another recommendation is to limit elephant numbers according to the space available at each camp and adjoining land. Keeping elephant numbers in proportion to the space could allow management to provide longer chains (20–30 m) providing more freedom of movement. Thus, we suggest it is important to manage appropriate numbers of elephants suitable for the natural environment, with responsible mahouts to care for them by encouraging daily exercise and good quality food. Opportunities to socialize with compatible elephants should be provided, even in restricted areas. These adaptations could ensure better welfare for elephants, not just during this pandemic, but going forward once tourism returns to pre-pandemic levels, and in anticipation of future crises.

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 authors.

Ethics statement

The studies involving human participants were reviewed and approved by Faculty of Veterinary Medicine, Chiang Mai University Research Ethics Committee (HS1/2564). The patients/participants provided their written informed consent to participate in this study. The animal study was reviewed and approved by the Institutional Animal Care and Use Committee, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand (FVM-ACUC, permit number S4/2564). Written informed consent was obtained from the owners for the participation of their animals in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

JS conceived and designed the experiments, performed the experiments, analyzed the data, contributed

reagents/materials/analysis tools, prepared figures and/or tables, authored, reviewed drafts of the paper, and approved the final draft. JB conceived and designed the experiments, contributed reagents/materials/analysis tools, funding acquisition, reviewed drafts of the paper, and approved the final draft. PB conceived and designed the experiments, performed the experiments, contributed reagents/materials/analysis tools, and approved the final draft. CT performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, funding acquisition, and approved the final draft. VP analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, and approved the final draft. JK conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, contributed reagents/materials/analysis tools, funding acquisition, project administration, authored, reviewed drafts of the paper, and approved the final draft. All authors contributed to the article and approved the submitted version.

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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.2022.1038855/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Examples of camp management and mahout routine work during COVID-19. **(A)** Elephant in a nearby forest, **(B)** Walking activity, **(C)** Elephant at coffee café, **(D)** Covered shed with elephants chained near each other **(E)** Bathing elephant by mahout **(F)** Supplement food from private donations. Photography by Jarawee Supanta.

SUPPLEMENTARY FIGURE 2
Changes in the number of visitors **(A)**, elephants **(B)**, mahouts **(C)** and other staff **(D)** at individual elephant tourist camps in northern Thailand before (T00) and through six survey periods [T01 (April–August, 2020), T02 (September–December, 2020), T3 (January–April, 2021), T04 (May–August, 2021), T05 (September–December, 2021) and T06 (January–April, 2022)] during the COVID-19 pandemic and corresponding reduction in tourist numbers.

SUPPLEMENTARY FIGURE 3
Variety of roughage **(A)** and supplement **(B)** foods offered to elephants at camps in northern Thailand before (T00) and through six survey periods [T01 (April–August 2020), T02 (September–December, 2020), T3 (January–April, 2021), T04 (May–August, 2021), T05 (September–December, 2021) and T06 (January–April, 2022)] during the COVID-19 pandemic. Data represent the percentage of camps offering each food type across time periods.

SUPPLEMENTARY FIGURE 4
Mahout activities of the daily routine at individual elephant tourist camps in northern Thailand through six survey periods [T01 (April–August, 2020), T02 (September–December, 2020), T3 (January–April, 2021), T04 (May–August, 2021), T05 (September–December, 2021) and T06 (January–April, 2022)] during the COVID-19 pandemic.

SUPPLEMENTARY TABLE 1
Questionnaire of Project: An assessment of the elephant camp management in the COVID-19 crisis for better health on elephant welfare in Chiang Mai tourist industry. The full questionnaire sheet used to record information during camp visits.

SUPPLEMENTARY DATA
The raw data.

References

1. Škare M, Soriano DR, Porada-Rochoń M. Impact of COVID-19 on the travel and tourism industry. *Technol Forecast Soc Change.* (2021) 163:120469. doi: 10.1016/j.techfore.2020.120469

2. Huynh DV, Truong TTK, Duong LH, Nguyen NT, Dao GVH, Dao CN. The COVID-19 pandemic and its impacts on tourism business in a developing city: insight from Vietnam. *Economies.* (2021) 9:172. doi: 10.3390/economies9040172

3. Rasul G, Nepal AK, Hussain A, Maharjan A, Joshi S, Lama A, et al. Socio-economic implications of COVID-19 pandemic in south Asia: Emerging risks and growing challenges. *Front Sociol.* (2021) 6:629693. doi: 10.3389/fsoc.2021.629693

4. Mandel A, Veetil V. The economic cost of COVID lockdowns: an out-of-equilibrium analysis. *Econ Disasters Clim Change.* (2020) 4:431–51. doi: 10.1007/s41885-020-00066-z

5. Haryanto T. Editorial: Covid-19 pandemic and international tourism demand. *JDE J Dev Econ.* (2020) 5:1–4. doi: 10.20473/jde.v5i1.19767

6. Shakya, Martina. *Risk, Vulnerability and Tourism in Developing Countries: The Case of Nepal, Vol. 56.* Berlin: Logos Verlag Berlin GmbH (2009).

7. Gössling S, Scott D, Hall CM. Pandemics, tourism and global change: a rapid assessment of COVID-19. *J Sustain Tour.* (2021) 29:1–20. doi: 10.1080/09669582.2020.1758708

8. Ismael NB, Sorguli S, Aziz HM, Sabir BY, Hamza PA, Gardi B, et al. The Impact of COVID-19 on small and medium-sized enterprises in Iraq. *Ann Romanian Soc Cell Biol.* (2021) 25:2496–505.

9. Climate Change. Greenhouse gas build-up reached new high in 2020. BBC News. (2021). Available online at: https://www.bbc.com/news/science-environment-59016075 (accessed August 20, 2022).

10. Pollution made the pandemic worse, but lockdowns clean the sky. *Science.* (2020). Available online at: https://www.nationalgeographic.com/science/article/pollution-made-the-pandemic-worse-but-lockdowns-clean-the-sky (accessed August 20, 2022).

11. Baptista J, Blache D, Cox-Witton K, Craddock N, Dalziel T, de Graaff N, et al. Impact of the COVID-19 pandemic on the welfare of animals in Australia. *Front Vet Sci.* (2021) 7:621843. doi: 10.3389/fvets.2020.621843

12. Usui R, Sheeran LK, Asbury AM, Blackson M. Impacts of the COVID-19 pandemic on mammals at tourism destinations: a systematic review. *Mammal Rev.* (2021) 51:492–507. doi: 10.1111/mam.12245

13. Williams JM, Randle H, Marlin D. COVID-19: Impact on United Kingdom horse owners. *Animals.* (2020) 10:1862. doi: 10.3390/ani10101862

14. Gomes-Neves E, Marques S, Alves-Pereira A, Osório P, Müller A, Baptista CS. Impact of COVID-19 restrictions in Portugal: a questionnaire to municipal and animal association shelters. *Animals.* (2021) 11:2532. doi: 10.3390/ani11092532

15. Newsome D. The collapse of tourism and its impact on wildlife tourism destinations. *J Tour Futur.* (2020) 7:295–302. doi: 10.1108/JTF-04-2020-0053

16. Buckley RC, Castley JG, Pegas F de V, Mossaz AC, Steven R. A population accounting approach to assess tourism contributions to conservation of IUCN-Redlisted mammal species. *PLoS One.* (2012) 7:e44134. doi: 10.1371/journal.pone.0044134

17. These locked-down cities are being reclaimed by animals. *World Economic Forum.* (2022) Available online at: https://www.weforum.org/agenda/2020/04/covid-19-cities-lockdown-animals-goats-boar-monkeys-zoo/ (accessed August 20, 2022).

18. Starving monkeys fed with watermelon as coronavirus halts tourism in Thailand. (2020). Available online at: https://uk.news.yahoo.com/starving-monkeys-fed-watermelon-coronavirus-150000828.html?guccounter=1&guce_referrer=aHR0cHM6Ly9hcGkuZ2RlbHRwcm9qZWN0Lm9yZy9hcGkvdjIvZG9jL2RvYz9mb3JtYXQ9aHRtbCZzdGFydGRhdGV0aW1lPTIwMTcwMTAxMDAwMDAwJmVuZGRhdGV0aW1lPTIwMjAwNDI3MjM1OTU5JnF1ZXJ5PXRvdXJpc20lMjBhbmltYWxzJTIwJTIyQ09WSUQtMTklMjAmbW9kZT1hcnRsaXN0Jm1heHJlY29yZHM9NzUmc29ydD1oeWJyaWRyZWw&guce_referrer_sig=AQAAALH7FWBnRXwlA226s3Ivetg7Fs7cCcAFvz1sZEowgpPMzPdNZu_QKSkG86AI-VhXsQ0vQDO5VqgL6h1AjJR1rLF4cV7eX6Ca-rfnv6wXz2IrlwG6TUUQtmAtwm-qrNN_vgJka5Jo5F3TOVdqjo8Pdo9lWsZpBRbhhx5SVHS6l2qZ (accessed August 25, 2022).

19. Sri Lanka's COVID-19 lockdown sets wildlife free but raises poaching threat. Mongabay Environmental News. (2020). Available online at: https://news.mongabay.com/2020/04/sri-lankas-covid-19-lockdown-sets-wildlife-free-but-raises-poaching-threat/ (accessed September 2, 2022).

20. Asian Elephant Specialist Group. *Asian elephant range states meeting, final report.* (2022) Available online at: http://www.asesg.org/PDFfiles/2017/AsERSM%202017_Final%20Report.pdf (accessed September 2, 2022).

21. IUCN Asian Elephant Specialist Group (AsESG), UPSC. BYJUS. (2021) Available online at: https://byjus.com/free-ias-prep/asesg-asian-elephant-specialist-group-iucn/ (accessed September 6, 2022).

22. Godfrey A, Kongmuang C. Distribution, demography and basic husbandry of the Asian elephant in the tourism industry in northern Thailand. (2009). Available online at: https://www.semanticscholar.org/paper/dayistribution%2C-Demography-and-Basic-Husbandry-of-the-Godfrey-Kongmuang/fc8723e66ecb48906621a10807f21d1404f501e3 (accessed June 27, 2022).

23. Bansiddhi P, Brown J, Thitaram C, Punyapornwithaya V, Somgird C, Edwards K, et al. Changing trends in elephant camp management in northern Thailand and implications for welfare. *PeerJ.* (2018) 6:e5996. doi: 10.7717/peerj.5996

24. Norkaew T, Brown J, Thitaram C, Bansiddhi P, Somgird C, Punyapornwithaya V, et al. Associations among tourist camp management, high and low tourist seasons, and welfare factors in female Asian elephants in Thailand. *PLoS ONE.* (2019) 14:e0218579. doi: 10.1371/journal.pone.0218579

25. Norkaew T, Brown J, Bansiddhi P, Somgird C, Thitaram C, Punyapornwithaya V, et al. Body condition and adrenal glucocorticoid activity affects metabolic marker and lipid profiles in captive female elephants in Thailand. *PLoS ONE.* (2018) 13:e0204965. doi: 10.1371/journal.pone.0204965

26. Bansiddhi P, Nganvongpanit K, Brown J, Punyapornwithaya V, Pongsopawijit P, Thitaram C. Management factors affecting physical health and welfare of tourist camp elephants in Thailand. *PeerJ.* (2019) 7:6756. doi: 10.7717/peerj.6756

27. Bansiddhi P, Brown J, Khonmee J, Norkaew T, Nganvongpanit K, Punyapornwithaya V, et al. Management factors affecting adrenal glucocorticoid activity of tourist camp elephants in Thailand and implications for elephant welfare. *PLoS ONE.* (2019) 14:e0221537. doi: 10.1371/journal.pone.0221537

28. WHO Thailand. *World Health Organization.* (2022) Available online at: https://www.who.int/thailand (accessed October 9, 2022).

29. Gairhe K. Veterinary care and breeding of elephants in Nepal. *Gajah.* (2012) 37:27–30.

30. Tang R, Li W, Zhu D, Shang X, Guo X, Zhang L. Raging elephants: effects of human disturbance on physiological stress and reproductive potential in wild Asian elephants. *Conserv Physiol.* (2020) 8:coz106. doi: 10.1093/conphys/coz106

31. Bansiddhi P, Brown JL, Thitaram C. Welfare assessment and activities of captive elephants in Thailand. *Animals.* (2020) 10:919. doi: 10.3390/ani10060919

32. Holdgate MR, Meehan CL, Hogan JN, Miller LJ, Soltis J, Andrews J, et al. Walking behavior of zoo elephants: Associations between GPS-measured daily walking distances and environmental factors, social factors, and welfare indicators. *PLoS ONE.* (2016) 11:e0150331. doi: 10.1371/journal.pone.0150331

33. Rowell ZE. Locomotion in captive Asian elephants (*Elephas maximus*). *J Zoo Aquar Res.* (2014) 2:130–5. doi: 10.19227/jzar.v2i4.50

34. Estimates of locomotion in Asian elephants *Elephas maximus* using video monitoring at Dublin Zoo, Ireland. *J Zoo Aqua Res.* (2021) 9:124–33. doi: 10.19227/jzar.v9i2.502

35. Varma S, Verghese G, Sarma KK, Kalaivanan N. *Captive elephants in forest camps of India.* (2010) Available online at: https://dayataspace.princeton.edu/handle/88435/daysp01tt44pq34v (accessed September 6, 2022).

36. Behavior and ecology of the Asiatic elephant in southeastern Ceylon. (2022) Available online at: https://repository.si.edu/handle/10088/5383 (accessed October 23, 2022).

37. Kurt F. Remarks on the social structure and ecology of the Ceylon elephant in the Yala National Park. In: Geist V, Walther F, editors. *The Behaviour of Ungulates and Its Relation to Management*. Cham: IUCN Publications (1974). p. 618–34.

38. Ortega J, Eggert L. Sukumar, R. *The Living Elephants: Evolutionary Ecology, Behavior, and Conservation*. New York, NY: Oxford University Press (2003). 478 p.

39. Morfeld KA, Meehan CL, Hogan JN, Brown JL. Assessment of body condition in African (*Loxodonta africana*) and Asian (*Elephas maximus*) elephants in North American Zoos and management practices associated with high body condition scores. *PLoS ONE.* (2016) 11:e0155146. doi: 10.1371/journal.pone.0155146

40. Norkaew T, Brown J, Bansiddhi P, Somgird C, Thitaram C, Punyapornwithaya V, et al. Influence of season, tourist activities and camp management on body condition, testicular and adrenal steroids, lipid profiles, and metabolic status in captive Asian elephant bulls in Thailand. *PLoS One.* (2019) 14:e0210537. doi: 10.1371/journal.pone.0210537

41. Forest Industry Organization. First baby elephant training ceremony. In: *Mahout and Thai Elephant Development Education College*. National Elephant Institute (2005). Available online at: http://www.fio.co.th/p/km/dayocument/54/54_KM_4_01.pdf (accessed September 2, 2021).

42. Grandin T. Safe handling of large animals. *Occup Med Phila Pa.* (1999) 14:195–212.

43. Magda S, Spohn O, Angkawanish T, Smith DA, Pearl DL. Risk factors for saddle-related skin lesions on elephants used in the tourism industry in Thailand. *BMC Vet Res.* (2015) 11:117. doi: 10.1186/s12917-015-0438-1

44. Kongsawasdi S, Brown JL, Boonprasert K, Pongsopawijit P, Wantanajittikul K, Khammesri S, et al. Impact of weight carriage on joint kinematics in Asian elephants used for riding. *Animals.* (2021) 11:2423. doi: 10.3390/ani1108 2423

45. Kiley-Worthington M. The welfare of performing animals. A historical perspective. *Anim Open Access J MDPI.* (2016) 6:76. doi: 10.3390/ani6110076

46. Rault JL. Friends with benefits: social support and its relevance for farm animal welfare. *Appl Anim Behav Sci.* (2012) 136:1–14. doi: 10.1016/j.applanim.2011.10.002

47. DeVries AC, Glasper ER, Detillion CE. Social modulation of stress responses. *Physiol Behav.* (2003) 79:399–407. doi: 10.1016/S0031-9384(03)00152-5

48. Plotnik JM, Waal FBM de. Asian elephants (*Elephas maximus*) reassure others in distress. *PeerJ.* (2014) 2:e278. doi: 10.7717/peerj.278

49. Buckley C. (2008). Captive elephant foot care: natural habitat husbandry techniques. In: Csuti B, Sargent EL, Bechert US, editors. *The Elephant's Foot: Prevention and Care of Foot Conditions in Captive Asian and African Elephants* (Ames: Iowa State University Press), p. 53–6.

50. Miller MA, Hogan JN, Meehan CL. Housing and demographic risk factors impacting foot and musculoskeletal health in African elephants (*Loxodonta africana*) and Asian elephants (*Elephas maximus*) in North American Zoos. *PLoS One.* (2016) 11:e0155223. doi: 10.1371/journal.pone.0155223

51. Fuktong S, Yuttasaen P, Punyapornwithaya V, Brown JL, Thitaram C, Luevitoonvechakij N, et al. A survey of stereotypic behaviors in tourist camp elephants in Chiang Mai, Thailand. *Appl Anim Behav Sci.* (2021) 243:105456. doi: 10.1016/j.applanim.2021.105456

52. Schmid J. Keeping circus elephants temporarily in paddocks – The effects on their behaviour. *Anim Welf.* (1995) 4:87–101.

53. Gruber T, Friend T, Gardner JM, Packard J, Beaver B, Bushong D. Variation in stereotypic behavior related to restraint in circus elephants. *Zoo Biol.* (2000) 19:209–21. doi: 10.1002/1098-2361(2000)19:3<209::AID-ZOO4>3.0.CO;2-7

54. Phuangkum P. *Elephant Care Manual for Mahouts and Camp Managers*. Bangkok: FAO Regional Office for Asia and the Pacific (2005). p. 172.

55. Varadharajan V, Krishnamoorthy T, Nagarajan B. Prevalence of stereotypies and its possible causes among captive Asian elephants (*Elephas maximus*) in Tamil Nadu, India. *Appl Anim Behav Sci.* (2016) 174:137–46. doi: 10.1016/j.applanim.2015.10.006

56. Bishop J, Hosey G, Plowman, A. *Handbook of Zoo Research, Guidelines for Conducting Research in Zoos*. London: British and Irish Association of Zoos and Aquariums (2013). 218 p.

57. Elephants in Thailand facing starvation as COVID-19 kills tourism. Available online at: http://nypost.com/2020/03/31/elephants-in-thailand-facing-starvation-as-covid-19-kills-tourism/ (accessed October 21, 2022).

58. Vanitha V, Thiyagesan K, Baskaran N. Food and feeding of captive Asian Elephants (*Elephas maximus*) in the three management facilities at Tamil Nadu, South India. *Sci Trans Environ Technov J.* (2008) 2:87–97. doi: 10.20894/STET.116.002.002.005

59. Kamal PG. Elephant status in Nepal. Updating the status of Asian elephant during COVID-19 pandemic. In: *2nd International Conference of Elephant and Wildlife Health Management in Asia*, 11–12 (2011).

60. Coleman GJ, Hemsworth PH, Hay M, Cox M. Modifying stockperson attitudes and behaviour towards pigs at a large commercial farm. *Appl Anim Behav Sci.* (2000) 66:11–20. doi: 10.1016/S0168-1591(99)00073-8

61. Hemsworth PH. Human–animal interactions in livestock production. *Appl Anim Behav Sci.* (2003) 81:185–98. doi: 10.1016/S0168-1591(02)00280-0

62. Hemsworth P, Barnett JL, Coleman G. The integration of human-animal relations into animal welfare monitoring schemes. *Anim Welf.* (2009) 18:335–45.

63. Crawley JAH, Lahdenperä M, Seltmann MW, Htut W, Aung HH, Nyein K, et al. Investigating changes within the handling system of the largest semi-captive population of Asian elephants. *PLoS One.* (2019) 14:e0209701. doi: 10.1371/journal.pone.0209701

64. Hart LA. The Asian elephants-driver partnership: the drivers' perspective. *Appl Anim Behav Sci.* (1994) 40:297–312. doi: 10.1016/0168-1591(94)90070-1

65. Mumby HS. Mahout perspectives on Asian elephants and their living conditions. *Animals.* (2019) 9:879. doi: 10.3390/ani9110879

66. Liehrmann O, Crawley JAH, Seltmann MW, Feillet S, Nyein UK, Aung HH, et al. Handler familiarity helps to improve working performance during novel situations in semi-captive Asian elephants. *Sci Rep.* (2021) 11:15480. doi: 10.1038/s41598-021-95048-w

67. Carlstead K, Paris S, Brown JL. Good keeper-elephant relationships in North American zoos are mutually beneficial to welfare. *Appl Anim Behav Sci.* (2019) 211:103–11. doi: 10.1016/j.applanim.2018.11.003

68. Vanitha V, Thiyagesan K, Baskaran N. Socio-economic status of elephant keepers (mahouts) and human–captive elephant conflict: a case study from the three management systems in Tamil Nadu, Southern India. *Gajah.* (2009) 30:8–12.

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Cage egg producers' perspectives on the adoption of cage-free systems in China, Japan, Indonesia, Malaysia, Philippines, and Thailand

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Asia is responsible for ~60% of global egg production. As in most of the world, nearly all of the egg-laying hens are housed in cages. While there is growing demand for cage-free eggs in many regions of the world, challenges have been reported when transitioning to these systems, which may affect the willingness of producers to transition. The aim of this research was to investigate the views of Asian egg producers on the feasibility of cage-free systems and what they perceive to be the main challenges and proposed solutions in adopting cage-free systems. A total of 224 egg producers (165 cage egg producers) completed questionnaires containing a mix of free-form, Likert scale and demographic items. Data were analyzed using thematic qualitative analysis and descriptive quantitative statistics. Responses indicated that cages are primarily used for their efficiency and ease of management. The most common reasons to consider adopting cage-free systems included improved animal welfare, increased market access, and increased product quality. A majority of producers (65%) responded "yes" or "maybe" when asked if they consider cage-free systems to be feasible in their country. Perceived challenges in adopting cage-free systems included reduced profitability, higher costs, and biosecurity and disease. Potential solutions included the development of the cage-free industry and market development. Most producers (72%) said more support is needed to establish cage-free farms, mostly pertaining to technical advice, training and resources. The findings of this study provide an enhanced understanding of the egg industry in these countries and potential areas for producer support in transitioning to cage-free systems.

KEYWORDS

animal welfare, chickens, hens, egg, production, Asia

Introduction

As of 2018, the continent of Asia was responsible for the production of 822 billion chicken eggs annually; 60% of total world production and was home to at least 3.1 billion egg-laying chickens (1). As is the case in most areas of the world, almost all of the hens are kept in cage production systems (1–3). Chicken and egg production arguably began in Asia, with the domestication of jungle fowl in natural open range farming environments (4). The industrialization of animal agriculture, coupled with the need to provide protein for growing populations, has facilitated the growth of the egg industry, unrivaled in the rest of the world.

Constituents and consumers around the world increasingly care about animal welfare and expect improved treatment and conditions for farm animals (5). Since the intensification of animal agriculture and the rise of affluence in Asia, widespread domestic poverty in countries such as China is rapidly becoming an epidemic of the past (6). Recent research has shown that "animal welfare" and "animal protection" are considered important in many countries in Asia amongst the general public (7–9), in agricultural science (10), and the livestock industry (11). One of the few studies on this topic that was conducted in the region, found that livestock industry leaders across Asia see a variety of benefits in improving animal welfare, such as; improved productivity, improved product quality, and market differentiation (12), and another study indicated that engaging industry stakeholders could be effective in improving industry practices and animal welfare (13).

There is growing demand for cage-free eggs from food businesses and consumers in Asia, and producers are looking to meet this demand by adopting cage-free systems (14). As such, cage-free egg production systems are currently emerging across the region (15). However, Asia and other regions of the world still primarily utilize cage-based systems of egg production; ∼90% of eggs produced in China, 80% in India, and almost 100% of eggs produced in Malaysia are produced in cages (9). The risk of negative economic implications, such as an increase in the cost of production resulting in higher egg prices (16), and a perceived reduction in the hygiene of cage-free eggs (17), could serve to undermine the transition to cage-free systems. A recent study in China supports this presumption, where cage egg producers considered that a transition to cage-free systems would represent a financial loss (3). The exact nature of financial implications and challenges to the perspective of egg producers in China, and many other nations, is yet to be investigated and evaluated in any depth.

The primary goal for the present research was to investigate, from the producers' perspective, the perceived feasibility of cage-free systems as well as the main challenges egg producers face in adopting and maintaining cage-free egg farms, and some potential solutions across six key countries in the region: China, Indonesia, Japan, Malaysia, Philippines and Thailand. The key questions focused on: (1) the reasons to use conventional cage systems; (2) the perceived reasons to use cage-free systems; (3) whether cage-free systems are an option; (4) the perceived challenges in adopting cage-free systems; (5) potential solutions to the perceived challenges; (6) whether more support would be needed when adopting cage-free systems; (7) what support is needed; and (8) who should offer that support. The findings of this study are anticipated to provide an enhanced understanding of the industry in the focus countries and offer insight into potential areas for initiatives to support the egg industry in these countries in the transition from cage to cage-free systems of egg production.

Methods

Research ethics

This research was granted ethics approval through the University of Queensland Human Ethics Committee (#2020002225). Data collection was conducted between January and June 2021.

Participants

Egg producers were eligible to participate in this study if they nominated their consent on the questionnaire, met the criteria in Table 1, and were deemed to have a working knowledge of their operation. Eligibility criteria was based on samples deemed representative of local industries in each country, rather than analogous criteria across all countries, as the nature of egg production industries vary by country. The countries selected for investigation in this study were selected for this inherent diversity in nature of production, diversification of culture and geographic distribution around Asia. Efforts were made to harmonize criteria where the scale of the industry allows, however the scale of the industries in each country did not allow for this. For example, farms tend to be no more than 50,000 hens in Indonesia, as compared to farms that commonly start at a size of 50,000 hens in China. As this area has scarcely been researched, and there does not exist a central repository for information in relation to cage-egg farms in any of these countries, the size of farm that was considered respectively "representative" was ascertained through consultation with local experts in each instance. In this nature, the perceptions reported in this study are representative of local industries, and findings are commonly delineated by country. Where similarities are found across countries and represented as

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TABLE 1 Participant eligibility criteria.

Cage producers	Farm size	Representative of the size of cage farms in each country*
	Farming system	Conventional cages
	Role	Engaged in a role that has sufficient power within the organization to make or contribute to decisions on transitioning to cage-free, and knowledge of the operation.
	Length of service	Must have been working within the industry for a minimum of 1 year.
Cage-free producers	Farm size	Minimum 10,000 hens
	Farming system	Any cage free system. If farms have both cage and cage-free operations, they will be interviewed as cage-free.
	Role	Engaged in a role that requires a technical awareness of on-farm operations, including the challenges and benefits of operating within the cage-free egg production system.
	Length of service	Must have been working within the industry for a minimum of 1 year.

*Industry representative sample by country, as below:

Country	Farm size (number of hens)
China	50,000+
Indonesia	10,000–50,000
Japan	500,000–1 million
Malaysia	50,000–500,000
Philippines	15,000–1 million
Thailand	50,000–500,000

aggregates, it could be considered that those perceptions may represent egg producers in Asia more broadly. Producers were approached by in-country academic collaborators (co-authors) based on their eligibility, which was ascertained by familiarly with their enterprise (including online research), and through network referrals. Eligibility and consent was re-established at the onset of participation in the study.

Research tool

Quantitative surveys are not always sufficient in investigating human attitudes and concerns (18) or in providing a "deeper" understanding of social phenomena (19). For this reason, a mixed methodology approach was adopted, with a primary emphasis on qualitative items.

Study information and an invitation to participate were prepared in local languages and sent *via* email to egg producers in China, Indonesia, Japan, Malaysia, Philippines, and Thailand. If the producers agreed to participate, they were provided with a link to an online questionnaire in their local language (Chinese, Bahasa Indonesia, Japanese, English, or Thai) to complete at a time that suited them. Responses were anonymous and were translated from the local language to English by translators proficient in each language for data analyses. Anonymity also served to protect data collected within this study, and raw and collated data were kept digitally and password protected. Separate questionnaires were developed for cage and cage-free producers, and the relevant questionnaire link was distributed depending on the production system used. A total of 20 questions, plus demographic and farm details, were asked across the questionnaires. Definitions of cage and cage-free production, as it pertains to this study, were offered to both cage and cage-free producers as follows;

Cage systems—The use of wire cages to house laying hens inside sheds.

Cage-free systems—Housing that does not use cages and in which the hens can move freely throughout a shed. Cage-free systems include free-range or indoor systems and can have one or more levels (aviaries).

The specific questions relevant to this paper asked the following:

- Most egg farmers in your country and around the world use cages. What are the reasons for using cages compared to cage-free systems? (Open-ended)
- Some egg farmers are changing to cage-free systems. What do you think are the reasons to use cage-free compared to cage systems? (Open-ended)
- Do you think cage-free systems are an option in your country? (Yes/No option)
- What do you think are the biggest challenges and problems that prevent cage farmers from using cage-free systems? (Open-ended)
- If an egg farmer in your country decided to use a cage-free system what would be some of the solutions to the challenges (outlined in Q4 above)?
- 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 option)
- What support would they need? (Open-ended)
- Who should offer that support? (Open-ended).

Data analysis

The data were compiled, coded and cleansed, whereby responses that were abandoned by participants were removed and data columns were aligned for all countries to correspond with each question. All responses that were translatable were included in the analysis. Binary and numerical data were summarized and qualitative data was subjected to manual thematic analysis by the corresponding author (M.S) using software packages Nvivo (20) and Microsoft Office, where themes and subthemes were coded and described. Themes were created through a process of manual familiarity with the data to identify and group responses that were similar. For example, data (i.e., responses) that centered around economic implications would be classified together under a theme labeled "economic implications." Data within each theme were then further analyzed to identify similarities, and where they existed they were grouped and labeled. For example, within the theme of "economic implications" some responses pertained to perceived expenses in operating cage-free systems as opposed to cage-based systems, and others pertained to a perceived inability to access a market for cage-free eggs that would compensate for any increased operational expenditure. Each of these would be considered sub-themes to "economic implications." In some instances, responses would be analyzed further again until the data were saturated and labeled into themes to the level in which all similar responses could be grouped, a level of detail as it existed could be reported, and all data were represented. The datapoints (i.e., responses) in each theme and subtheme were then quantified to understand the frequency and, therefore, emphasis according to the producers.

Results

A total of 224 Asian egg producers were successfully recruited into this study however 22 did not complete the questionnaire. Two hundred and two producers participated through to completion of the questionnaires. Of these, 165 were producers that operate cage systems, and 37 using cage-free systems. This paper focuses primarily on the responses of the cage producers, with an accompanying paper presenting the results of the cage-free producers on the challenges in maintaining cage-free systems, including on-farm operational challenges and the support needed by cage-free egg producers. The numbers of cage producers that participated from each country were opportunistic and were: China (22); Indonesia (103); Japan (10); Malaysia (8); Philippines (10); Thailand (12); with a total of 165 cage producers. Producers' responses are shown below each question, in the order in which they appeared in the questionnaire.

TABLE 2 Ranking of reasons for using cage systems rather than cage-free systems, by country (cage producers, $n = 165$).

	Themes—number of responses
China	• Reduce cost ($n = 9$) • Land optimization ($n = 5$) • Ease/convenience of management ($n = 5$) • Scalability ($n = 5$) • Staff costs ($n = 3$)
Indonesia	• Ease/convenience of management ($n = 45$) • General efficiency of resources ($n = 23$) • Land optimization ($n = 7$) • Increased productivity/yield ($n = 5$) • Staff costs ($n = 2$)
Japan	• Hygiene of product ($n = 6$) • Reduced costs ($n = 4$) • Ease/convenience of management ($n = 2$) • Increased productivity/yield ($n = 2$) • Biosecurity/disease transmission (specific emphasis on humidity and moisture mitigation; $n = 2$)
Malaysia	• Increased productivity/yield ($n = 4$) • Reduced cost ($n = 3$) • Ease/convenience of management ($n = 2$) • Land optimization ($n = 2$) • General efficiency of resources ($n = 2$)
Philippines	• Ease/convenience of management ($n = 7$) • Reduced cost ($n = 4$) • Land optimization ($n = 2$)
Thailand	• Reduced costs ($n = 4$) • Ease/convenience of management ($n = 3$) • Staff costs ($n = 2$)
All countries	• Ease/convenience of management ($n = 94$) • Reduced cost ($n = 24$) • Land optimization ($n = 22$) • Increased productivity/yield ($n = 20$) • General efficiency of resources ($n = 19$)

Themes were included when they appeared at least twice in responses within a country's data and were limited to the top five for each country.

Perceived reasons to use cage-based systems

"*Most egg farmers in your country and around the world use cages. What are the reasons for using cages compared to cage-free systems?*"

The convenience of operations and the reduction of costs were the most frequently cited reasons for using cage-based systems, as cited by producers. Summarized responses are listed per country in Table 2, and are displayed as an aggregate across countries in Figure 1.

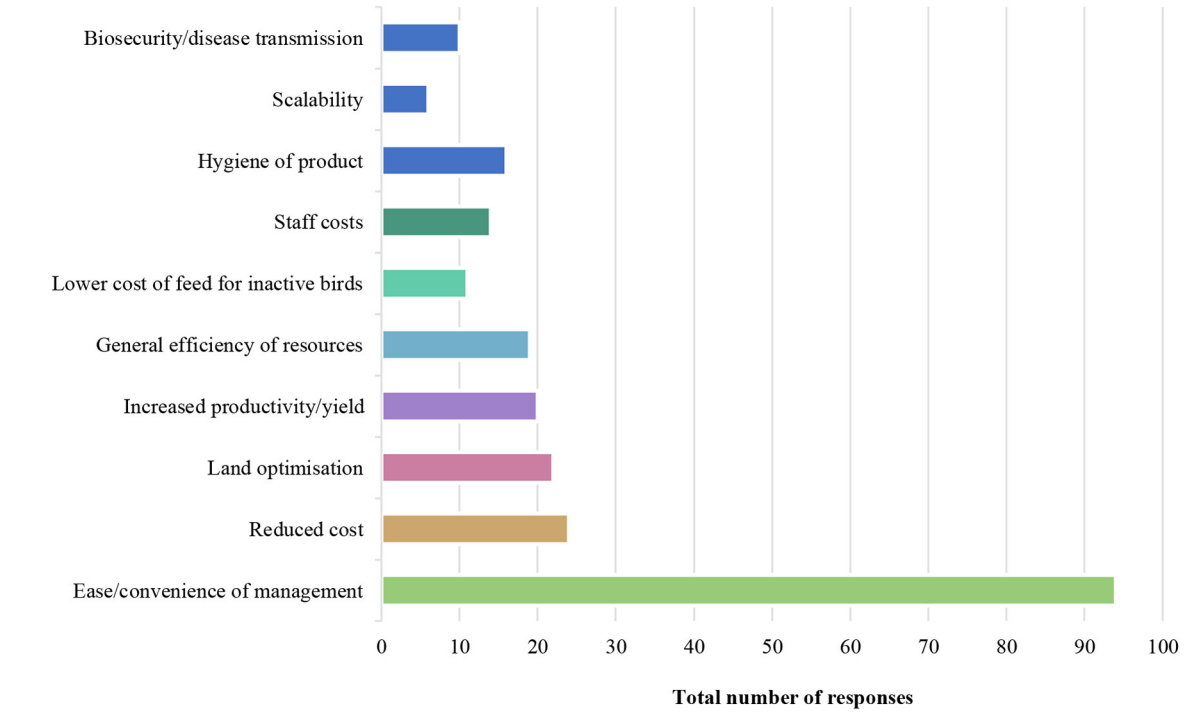

FIGURE 1
Cage egg producers' top 10 reasons for using cage over cage-free systems of production, displayed as the aggregate of data across all countries.

Perceived 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 compared to cage systems?*"

A total of 93.4% cage egg producers identified at least one reason to adopt cage-free systems. Improving bird welfare, gaining access to a wider market, and brand differentiation were the most frequently cited reasons producers identified for using cage-free systems of egg production. All reasons to consider adopting cage-free systems are ranked by frequency of appearance by country in Table 3, and visually displayed as an aggregate across the region in Figure 2.

Perceived feasibility of cage-free systems

"*Do you think cage-free systems are an option in your country? (Yes/No)*"

Across all countries, 24.8% of egg producers responded "Yes," 35.5% responded "No," and 40.6% responded "Maybe." The distribution of these responses, by country, are presented in Figure 3.

Barriers to adopting cage-free systems

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

A total of 217 barriers to moving to cage-free systems were identified by cage producers ($n = 165$). These barriers often represented recurring themes, predominantly centered around land availability, cost, management, and disease mitigation. The themes that emerged through the data, and their quantification, are visually summarized in Figure 4. Themes appearing in $> 2\%$ ($n \geq 4$) of responses were considered notable for inclusion during thematic analysis.

Solutions to adopting cage-free farms

"*If an egg farmer in your country decided to use a cage-free system what would be some of the solutions to the challenges (outlined above)?*"

Most commonly, industry development such as the application of technologies in improving on-farm practices

TABLE 3 Ranking of perceived reasons that cage-egg producers adopt cage-free systems in each country, including frequency of appearance of response (*n*) per country.

Country	Top responses of cage producers by number
China ($n = 28$)	• Improved animal welfare ($n = 7$) • Increasing buyer/consumer demand ($n = 5$) • Improved product quality ($n = 5$) • Access to higher-end market/higher price point ($n = 3$) • Access to government subsidy ($n = 2$)
Indonesia ($n = 53$)	• Improved animal welfare ($n = 31$) • Low investment cost ($n = 18$) • General cost saving ($n = 15$) • Management improvements ($n = 8$) • Improved bird health ($n = 4$)
Japan ($n = 14$)	• Higher price point ($n = 6$) • Increasing buyer/consumer demand ($n = 2$) • Brand marketing/differentiation ($n = 2$) • Improved animal welfare ($n = 2$)
Malaysia ($n = 12$)	• Improved animal welfare ($n = 4$) • Increasing buyer/consumer demand ($n = 3$) • Access to higher end market/higher price point ($n = 3$) • Brand marketing/differentiation ($n = 2$)
Philippines ($n = 25$)	• Improved animal welfare ($n = 8$) • Access to higher end market/higher price point ($n = 3$) • General cost saving ($n = 3$) • Access to humane "guilt-free" market ($n = 3$) • Access to "health food" market ($n = 2$) • Brand differentiation ($n = 2$)
Thailand ($n = 26$)	• Brand marketing/differentiation ($n = 7$) • Improved animal welfare ($n = 6$) • Access to international markets/keeping up with modern global practices/EU standards ($n = 3$)
All countries ($n = 158$)	• Improved animal welfare ($n = 59$) • Wider market access/increasing demand/brand ($n = 50$) • General cost saving ($n = 24$) • Product quality/price point ($n = 23$) • Low investment cost ($n = 20$)

Themes were included when they appeared at least three times within that country data and are limited to top 5 for each country.

and bird health in cage-free systems, along with market development, including demonstration that cage-free eggs can be sold at a higher price, were cited as solutions by egg producers. Quantification of the emerging themes is provided in Table 4, and the top themes are shown in relation to each other in Figure 5.

Support needed to adopt cage-free systems

"*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?*"

Across all countries, 72% responded "yes," 7% "maybe," and 22% "no."

"*What support would they need?*"

When asked to share their thoughts on the nature of support that is needed in considering adoption of cage-free systems of egg production, producers drew attention to the need for training, knowledge and access to experts in effective cage-free operations and bird health, along with financial assistance, including subsidies and capital, and market growth through consumer awareness. For example, one cage producer in Indonesia stated: "The government should eliminate the upper price limit because it can cause disincentive for farmers…farmers are threatened by operational licensing, and standard price is rarely evaluated based on the farm's budget and cost."

The themes of all responses across countries are quantified in Table 5, and an overview is presented visually in Figure 6.

"*Who should offer that support?*"

Egg producers most frequently identified their domestic government, and government departments within it (55%), as the stakeholder that should provide support. This was followed by the private sector (12%), then in equal part industry experts/consultants, industry and veterinary associations, and the farming and management network themselves. This data is presented in Table 6, and further illustrated in Figure 7.

Discussion

Challenges in adopting cage-free systems

The findings of this study present that the main reasons egg producers choose cage systems are centered around efficiency; that they are easier to operate and they reduce costs, while increasing the yield of eggs. Having not been exposed to a natural environment, the eggs are cleaner at collection, reducing

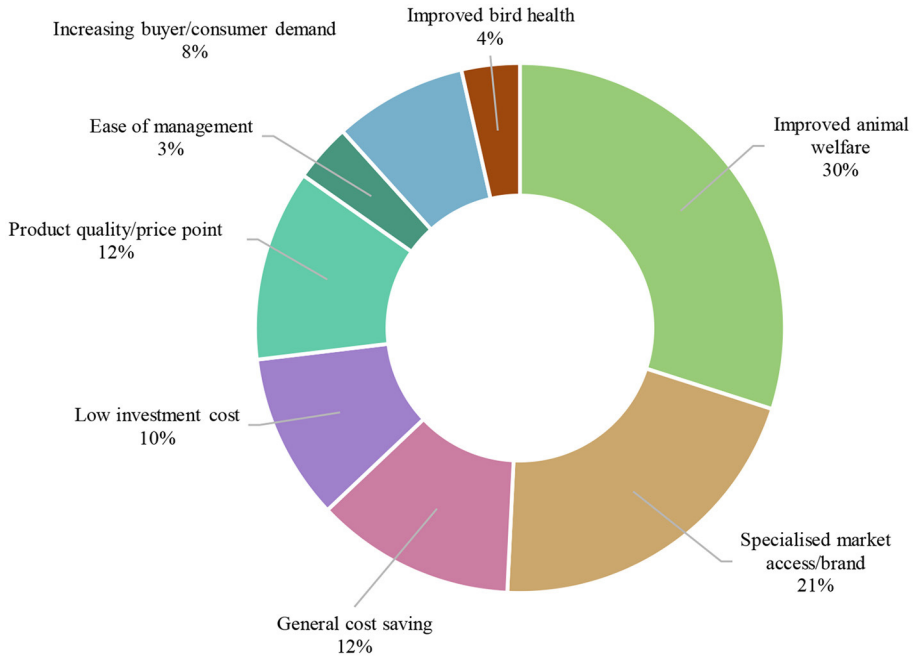

FIGURE 2
Cage egg producers' perceived reasons to adopt cage-free systems across all countries.

cleaning requirements. As is also often the case with intensive housing systems, another incentive for choosing cage-based systems is the ability to utilize land space for maximized output. These perceptions are in line with realities presented in the wider literature, that although relatively comparable in some conditions, cage systems were found to generally be more efficient. One rigorous study in the UK showed that while both cage and cage-free systems met production rate standards published by the National Farmers' Union (21), cage systems produced 5–7% more eggs in the span of a year; a study in Africa showed a difference of battery cage economic efficiency of 0.92 compared to 0.89 in a single level deep litter system (22), and an economic study in India also found efficiencies increased in cage systems (23). These increased efficiencies decrease operating costs. Another recent study in the USA demonstrated that aviary housing system (cage-free) operating costs were 23% higher than conventional cage (battery) systems, while the operating costs for enriched cage systems was 4% higher than conventional (battery) cage systems (24). Increased operating costs feed directly into the top challenge cage producers presented us with in considering a shift to cage-free systems: reduced profitability. On reviewing literature from Europe, North America and Australia, it appears that this is unsurprisingly also the primary reported barrier to transition to cage-free egg production in these regions. An economist's strict analysis of Californian egg prices after the ban of the sale of eggs from battery cage systems found that the prices of eggs increased, which resulted in higher prices and decreased consumer surplus (16). However, when adjusting for available data on the financial value of human altruism, and transversely also adding the transition cost to producers, another study found the opposite in the theoretical case of a nation-wide ban on cage-egg production in the USA; it found that benefits would far outweigh costs (25). Additionally, when considering higher operating costs, some losses could be associated with flock mortality and more generally, lack of experience with efficient cage-free operations. Demonstrating this, one more recent study conducted a meta-analysis of hen mortality across the various systems in 16 different countries over two decades, to find that as experience operating cage-free systems increased, mortality dropped an average of 0.35–0.65% annually, until there were no significant differences between the cage and cage-free production systems (26).

While the above studies are informative in relation to egg production in USA and Europe, they were not conducted in Asia, and findings may not be directly transferable. Agricultural factors often differ across and between regions; including breeds, climate, production systems, availability of farm resources, and other external factors such as the traits of the domestic markets, economic and geopolitical structures, and culture. In this region, the literature has been scarce, with few exceptions. One small but important exception conducted in-depth qualitative interviews with 15 cage egg producers in China. Resonating with findings in Europe and USA this study found that abandoning conventional

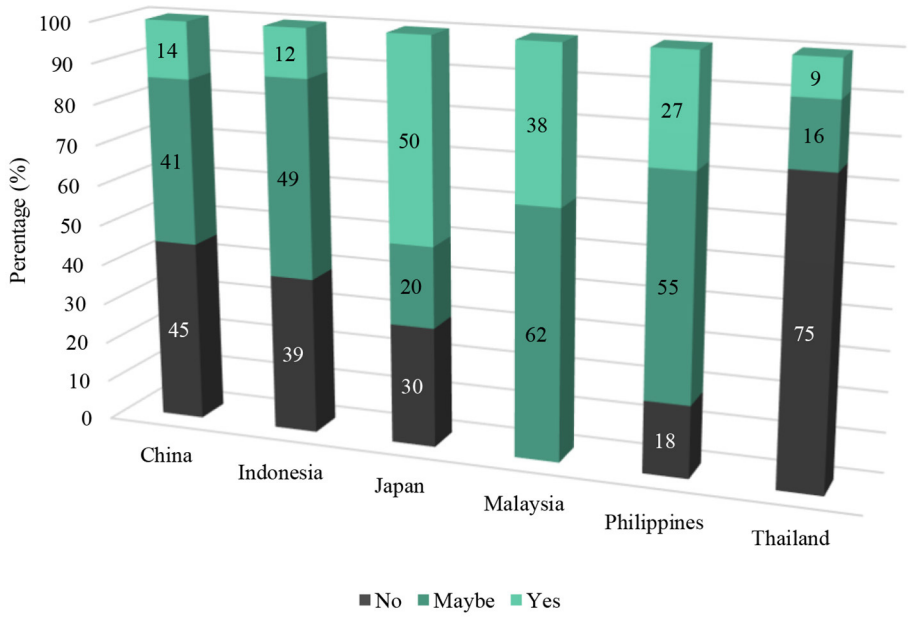

FIGURE 3
Cage egg producers' perceived feasibility of cage-free systems in their respective countries by percentage (%).

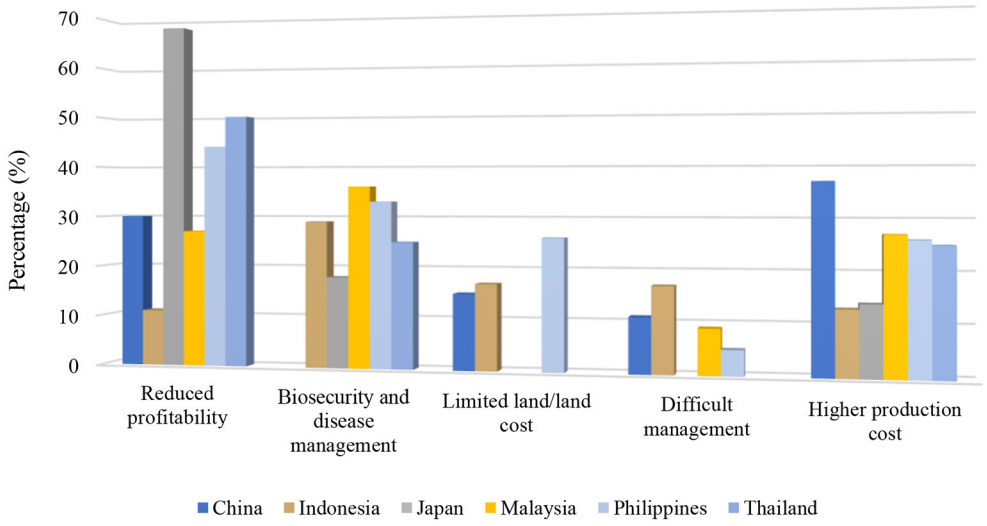

FIGURE 4
Cage egg producers' most frequently identified barriers to adopting cage-free systems displayed by country.

cages in favor of cage-free systems was considered a financial loss. When this perceived financial loss is coupled with a lack of domestic social pressure to adopt higher welfare systems, interest levels in transitioning to cage-free were unsurprisingly low (3). As echoed in the present findings, it remains that cage systems do present economic incentives to egg producers in Asian countries, as they do around the world. Still, there exists a growing trend to shift away from conventional cages in many global regions, and the current situation and perspectives in the focus countries may change in the coming years. Driving these key developments include domestic and international trends toward higher quality products, and increasing affluence in key states (27). While differences in operational costs and profitability can be demonstrated in present times, the growth

of markets willing to offset the welfare of hens, the increasing exposure and experience of producers in relation to cage-free systems, and even the potential for future legislative shifts that ban cage systems, could change this balance considerably. As states find themselves in increasingly comfortable economic positions and stages of development, animal welfare is of increasing concern to consumers (28, 29). In specific regard to the countries investigated within this study, recent research found that of egg consumers in China, Malaysia, Philippines and Thailand, ∼72, 73, 77, and 78% in each country respectively stated that it mattered to them that hens laying eggs do not suffer (30). Furthermore, 65, 69, 71, and 68% in China, Malaysia, Philippines and Thailand respectively went on to state that they would prefer to buy eggs from hens not kept in cages (30). This shift is also reflected in the multitude of global commitments from large multinational food companies to source cage-free eggs in their supply chains (14).

The second top challenge identified by producers in the present study, when considering the adoption of cage-free systems, was biosecurity and disease control. To support this, one study found that cage systems did slightly reduce the horizontal transmission of salmonella and campylobacter as compared to cage-free environments on wood shavings (as the shavings were considered to allow the disease to live longer) and cages with manure removal belts slightly reduce the bacteria count on eggs (17, 31). Importantly, however, there was no difference between bacteria on washed cage and cage-free eggs (17). It is important to note that the perspectives presented in this study are the producers' perceptions and are not indicative of consumer perceptions. One example of the potential disparity between perceptions in this study and consumer perceptions was "health benefits" of humans consuming cage eggs. While producers and cage proponents present that the easily monitored and maintained nature of harvesting eggs in cage systems reduces microbiological contact of eggs (32), consumers may instead associate organic, natural and high animal welfare with improved health benefits of the products from cage-free systems (33). Anecdotally, this is also the case with the use of native breeds and traditional farming methods in some areas of Asia, where consumers tend to perceive "naturalness" of these breeds as "healthier."

In the wider body of literature around challenges to bird health in egg production, destructive hen behaviors—such as feather pecking and cannibalism—are frequently featured, however, these behaviors were interestingly not presented with any significance by egg producers in this study.

Lastly, despite hosting a national land mass at least five times greater than any other country in this study, producers in China (15%) identified the availability of suitable land as a barrier to transitioning to cage-free systems. Most egg production in China (∼90%) is cage-based, at a scale seen no where else

TABLE 4 Frequency of perceived solutions to overcoming the aforementioned barriers that prevent cage farmers from using cage-free systems.

Emerging themes	Frequency
Land availability	
• Provision or purchase of an appropriate land area	21
• Establish farms further away from the business districts and prevent agricultural land conversation to residential	5
• Establish farms in appropriate environments/climates	5
Provision of support	
• Availability of financing/investors	13
• Affordable staff resourcing/Human Resources training	8
• Increase government subsidy/industry incentives	3
• Provision of nests and housing resources	4
• Equipment and maintenance	2
Market development	
• Price increase (eggs)	21
• Increase demand/consumption	9
• Demonstrate total increase profit in cage-free farming	7
• Standardize price for cage-free eggs	6
• Strengthen brand strategy/public relations/events	5
Industry development	
• Apply technology and innovation to develop improved on-farm practices (bird health and bird security)	20
• Demonstrate effective disease mitigation strategies/biosecurity/food safety	19
• Apply technology and innovation to develop improved general on-farm management practices (including feed distribution, flock sizes, and behavioral management)	16
• Knowledge increase/training for cage-free system planning/demonstrate benefits	13
• Increase productivity and feed conversion ratio	10
• Economic planning/sustainability	4
• Improve added value/quality of products	3
• Enriched cages or barns	3
• Restrict import eggs from overseas	2
• Limit volume of operation	1
• Major buyers take the lead	1
• Improved labeling	1
• Policy support	1
Societal facilitation	
• Community education (animal welfare, advantages and pricing)	6
• Introduction of legislation or regulation/all producers on the same system (incl grace period)	4
• More research/Investigate local alternatives that achieve the same results	3
• Continued GDP (gross domestic product)/economic growth	3
• Address more important issues first (i.e., antibiotic use)	1
Total	220

Displayed as the number of times the theme appeared in producers' answers.

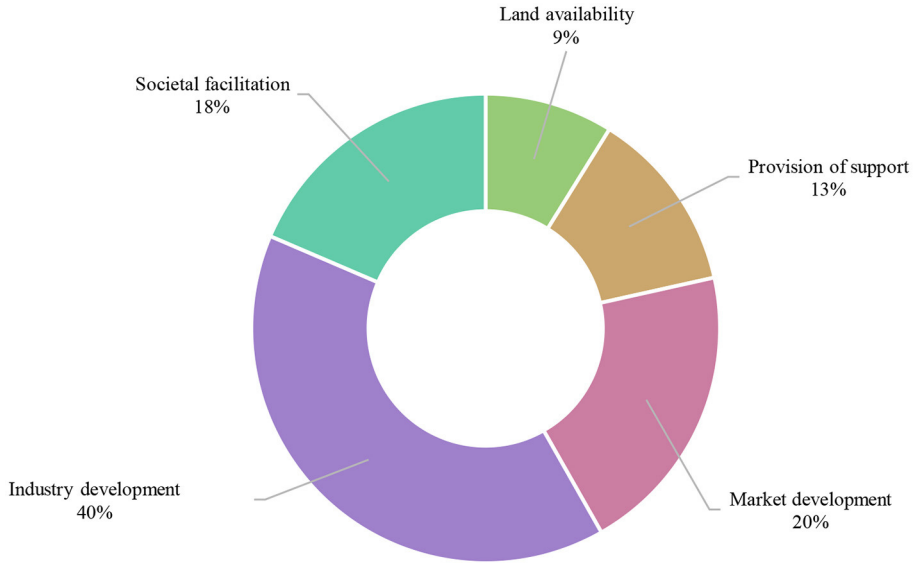

FIGURE 5
Egg producers' most frequently proposed solutions to the barriers preventing cage farmers from using cage-free systems.

in the world; ∼604.68 billion eggs per year (9). The nation also hosts the greatest population in the world; ∼1.4 billion people (34). It is possible that both of these factors impact egg producers' ability to envisage the quantity of chickens currently housed in cages being facilitated in cage-free ranges, alongside the human population.

Reasons to use cage-free systems

While the majority of egg producers across Asia still use cage systems, the findings of this study demonstrate that producers may be open to cage-free systems through acknowledgment of benefits for their use, and in majority, state that cage-free systems could be feasible in each country. When they were asked if cage-free systems were an option in their country, two-thirds of egg producers responded "yes" or "maybe," demonstrating a level of openness to cage-free systems. The one exception to this was Thailand, where 75% of producers did not believe cage-free systems to be feasible. The reasons for this were not revealed by this study, however Thailand has a thriving egg industry of over 94.8 million layer hens who are kept predominantly in cages (9).

Importantly, 93.4% of all respondents could identify at least one reason to adopt cage-free systems. The top benefits identified by egg producers in shifting to cage-free systems included improved animal welfare, access to wider markets, brand improvement, improved product quality, and reduced investment costs. While the animal welfare benefits in moving away from conventional cages are well-understood and accepted, additional beneficial aspects such as brand improvement, market widening, and increased sale price have also been demonstrated to grow as consumer awareness grows and cage-free systems become increasingly mandated by buying companies and their governments as a result (35). More broadly, Sinclair et al. (9) found that livestock industry leaders in Asia saw a number of benefits to improving the welfare of animals being farmed in general. These included improved productivity of the animals, improved product quality, reduction in disease, improved food safety and biosecurity, protection of natural resources, improved international trade opportunities, improved brand confidence, and options for increased revenue (9). Contrastingly, "cost savings" was broadly identified as a reason to adopt cage-free while "reduced profitability" was also identified as a challenge to adopting cage-free. In considering benefits more closely, a significant proportion of responses also explicitly identified the cost reduction element of establishing a cage-free farm, as compared to the expenditure required to install cage systems. It is therefore possible that the broader "cost savings" response in considering reasons to adopt cage-free farms, is also referencing this saved infrastructure expense. To consider the potential weighting of the reasons to operate cage vs. cage-free systems, Table 7 compared the top five findings against the results of a previous study with livestock industry stakeholders, which investigated and weighted the importance of general benefits of improving farm animal welfare (9).

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TABLE 5 Frequency of egg producers perception of the support that is needed when looking to adopt cage-free systems in across all countries.

Emerging theme	Emerging sub-themes	Frequency
Technical advice	Efficient operation and management/maintenance	15
	Controlling security/safety/health of birds	13
	Biosecurity/disease	10
	Brand marketing cage-free products	6
	Litter management	3
	Efficient farm layout and design	3
	Feeding management	3
	Shared experiences from other cage-free farmers	1
	Transition process	1
	Weather mitigation	1
Finance	Financial assistance/capital support/subsidies (including loan subsidies)	34
Provisions	Subsidized land (large/suitable)	13
	Staff/labor	12
	Bird provisions (feed, nests, medicine, and litter)	5
	Infrastructure (including roads and electricity)/equipment	4
Training/resources	Share knowledge/technical training for producers and personnel in effective cage-free management (continuous)	37
	Technical support/consultancy (including vets and government, mentors)	13
	Cost-benefit analysis/economic modeling	7
Market growth and accessibility	Grow cage-free market/consumer support through awareness (human health, organic, and animal welfare)	17
	Market accessibility/improve distribution channels (incl. reducing the price of distribution and joint marketing with other cage-free producers)	5
	Consumer acceptance of higher cage-free egg prices	3
Technological advances/upgrades	Advances in disease prevention and control on cage-free farms	5
	Efficiency/productivity upgrades	4
	Advances in egg hygiene/sanitation on cage-free farms	1
	System infrastructure upgrades (i.e., housing)	1
Governance	Law/regulation development	5
	Price regulation/standardization evaluation	5
	Reduced complexity of licensing, establishment of a certification body	3
	Full government support (tangibility, no favoritism)	3
	Policy support (including for trade)	2
Moral support	Understanding/support from the community and local farms (incl. reduced complaints)	4
	Reduce public criticism toward the industry	1

Solutions to the challenges

The top barriers for cage producers considering adopting cage-free systems, related to a perceived loss of profitability, increased direct and indirect costs—including disease—and a higher cost of production. This was not surprising, and is in line with literature from other areas of the world (16, 25, 36). The reduced efficiency and profitability that was perceived as a barrier to adopting cage-free farms is in part mitigated by the proposed solutions of development of the industry, market development and increased sales, and an increased price point. Coupled with market growth, improving the efficiencies of cage-free farms through training on best practices, technical advice, and investing to build cage-free efficiencies could also begin to address these challenges. These findings were echoed in a qualitative interview study conducted within China (3, 37), in which cage egg producers also suggested that increasing the domestic demand for higher welfare eggs through marketing, coupled with simultaneous ancillary measures such as exploring appropriate cage-free systems, and introducing regulation and producer training in cage-free system management, would provide solutions to producers desiring a transition to cage-free systems of egg production (3).

Although participants in the present study were not tested on their knowledge around cage and cage-free systems, some remarks and inconsistent responses provided by some cage egg producers could be interpreted as a lack of comprehensive understanding as to what constitutes a commercial cage-free farm (including barn and aviary systems). Awareness around what constitutes cage-free egg farms, and how they can operate effectively on a commercial scale, could be of foundational benefit. The perception of reduced control pertaining to bird

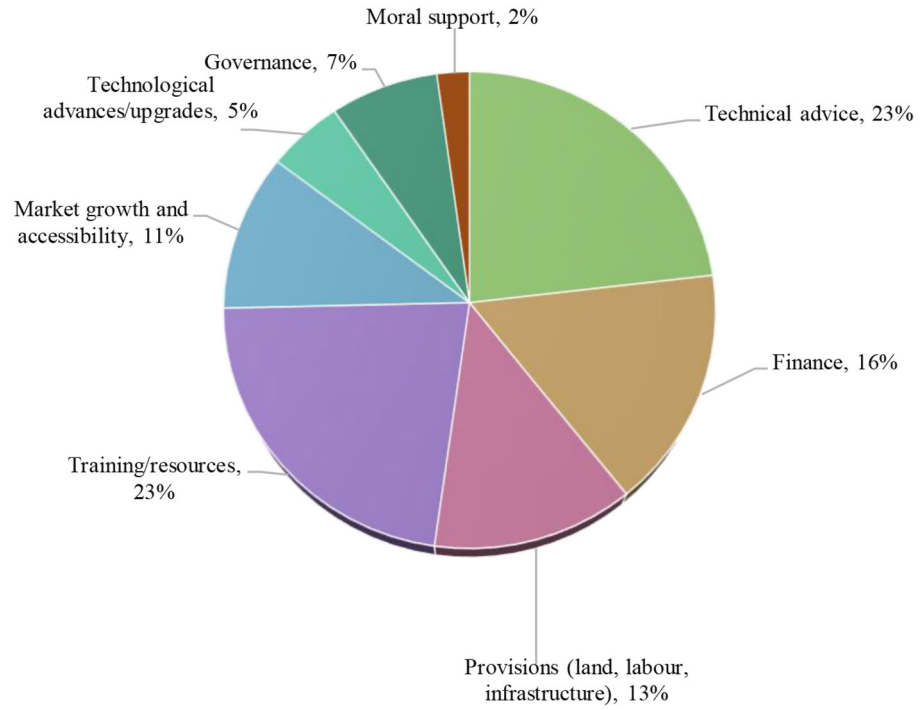

FIGURE 6
Egg producers' perception of the support needed to transition to cage-free systems, by emerging theme.

health and biosecurity, and the perceived reduced ability to prevent and treat disease, could also be addressed through the demonstration of model farming and biosecurity practices. In addition, applying technology and innovation to address bird health and biosecurity concerns were presented as solutions by producers, which could also be considered reasonable and practicable ways to mitigate concerns with a shift to cage-free systems. Further investigation to identify the specific technologies and technological development that were inferred by producers would be useful.

Support needed to adopt cage-free systems

Most producers believed that more support is needed to establish cage-free farms. Amongst the top types of support that were deemed needed were technical advice, training and resources. This reflects the findings by another recent study in the region, where livestock stakeholders presented that training and public awareness were amongst the solutions to wider animal welfare concerns for farmed animals (12). It is important to note that whilst cage-free systems offer opportunities to vastly improve animal welfare, they also present some challenges. As noted by one review, "improved animal welfare" needs refinement and consistency in practice; "welfare in cage-free systems is currently highly variable, and needs to be addressed by management practices, genetic selection, further research, and appropriate design and maintenance of the housing environment" (38).

In relation to identifying the key stakeholders from whom support is most needed should an adoption of cage-free systems be undertaken, "government," and specific government departments were identified in all countries, echoing the findings of earlier studies around motivational forces for animal welfare (11, 39), and international strategy (13). With the ability to provide guidance, resources, and to enact law and binding standards and policy, these findings reinforce the importance of government engagement, investment and, at a minimum, collaboration for any large-scale change to be sustainable.

Summary of animal welfare implications

The study provides an increased understanding of the egg industry in key Asian countries, as well as important solutions and support needed, nominated by egg producers themselves, when considering adopting cage-free systems of egg production. Since cage-free systems have the potential to enhance animal welfare, information that can be used to

TABLE 6 Frequency of egg producers' perceptions around who should be offering the support listed should they transition to cage-free systems.

Country	Responsible party	Frequency
China ($n = 23$)	Government	10
	Professional organizations/Industry	4
	Experts	3
	High end consumers/egg selling companies	2
	Overseas equipment suppliers	1
	Banks	1
	Other countries	1
	Technology service institutes	1
	Unsure	1
Indonesia	Government	48
($n = 107$)	Academics/institutions	10
	Community/everyone	10
	Related private sector (i.e., systems, bird feed companies, pharmaceutical companies, etc.)	9
	Farmers	8
	Vets/vet associations	5
	Nobody/unsure/unclear	5
	Industry associations	4
	Consultants/specialists	4
	Advocates	3
Japan ($n = 10$)	Government	9
	Nobody/unsure	3
	Private sector	1
	Other cage-free producers	1
	Media	1
Malaysia ($n = 9$)	Department of Veterinary Services (DVS)/Government	6
	Buyers/larger corporation	3
	Universities	1
	Farmers associations	1
	Equipment suppliers	1
	Poultry breeders	1
	Overseas experts	1
Philippines	Bureau of Animal Industry/Government	9
($n = 14$)	Nobody/unsure	2
	Equipment suppliers	2
	Management	1
	Banks	1
	Related private sector	1
	Other cage-free farmers	1
	Advocates	1
Thailand	Government (Animal Husbandry Department / Department of International Trade/Ministry of Agriculture, Commerce and Public Health)	14
($n = 15$)	Equipment suppliers	1
	Animal advocates	1
	Media	1
	Banks	1

improve the competitiveness of these systems and support egg producers is crucial.

Summary of the key results:

- The main reason producers choose to use cages—ease/convenience of management (53% of all responses)
- When cage producers were asked whether cage-free systems are a viable option, 35.5% said "no," 40.6% said "maybe," and 24.8% "yes," and 93% of cage producers identified at least one reason to adopt cage-free systems.
- The top four perceived reasons to go cage-free by cage producers included: animal welfare 30%, market access 21%, cost saving 12%, and product quality 12%.
- The top challenges preventing cage producers from adopting cage-free systems are; reduced profitability, biosecurity/disease, and higher cost of production.
- Top proposed solutions to these challenges are; development of the industry 40%, market development 20%, and societal facilitation 18%.
- Most producers believe more support is needed to establish a cage-free farm; 72% "yes," 7% "maybe," and 22% "no".
- The top types of support that is needed are; technical advice 23%, training/resources 23%, and provisions 13%.
- The top stakeholder that producers nominated that should provide support was the government, in 55% of responses.

Applications

The findings of this study provide a basis with which to engage with egg producers in the focus countries. In the absence of reformative laws, there exists a need to increase the competitiveness of cage-free systems, and an increase in the perceived benefits in favor of cage-free systems. This is particularly the case regarding efficiency and management processes.

Initiatives aimed at supporting the egg industry through training, knowledge dissemination, and financial assistance may have an increased likelihood of engagement with producers in Asia. Some existing programs applied in other areas of the world could be usefully tailored and introduced to Asia. Examples of this could include Hennovation in Europe; "practice-led innovation supported by science and market-driven actors in the laying hen and other livestock sectors" (40), and the establishment of government partnered industry-based training centers.

Further research quantifying the strengths of the reasons to transition to cage-free systems identified by egg producers in this study could be conducted, as could rigorous efficiency comparisons and economic modeling for best practice operated farms of both systems, in the context of local conditions and breeds.

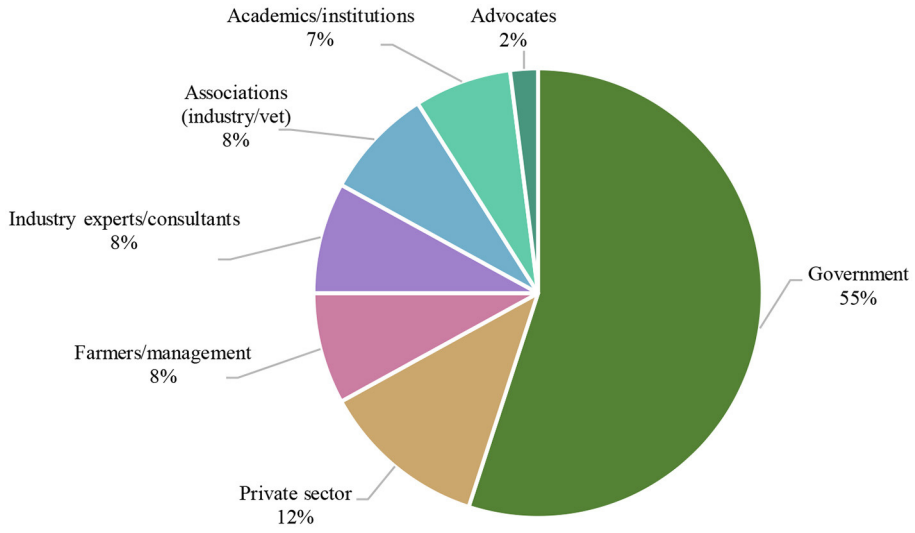

FIGURE 7
Egg producers' perception of the stakeholders that are most frequently deemed as required to provide support for transitioning to cage-free systems by percentage.

Informed by the key barriers and solutions presented by egg producers in this study, we suggest potential initiatives to support the transition to cage-free egg production in Asia. Some of the listed potential initiatives may be more strongly supported in certain countries. It is important to note that prior to introducing any of the suggested initiatives, further research should be conducted as to the suitability, feasibility, and approach. In considering the findings of this study, regarding perceived stakeholder support, it is also recommended that initiatives partner with government and the local industry wherever possible. Further research with a wider range of expert stakeholders associated with Asian egg industries (poultry experts such as veterinarians, ethologists, housing, climate and management specialists, nutritionists, breeding companies, along with legal, food safety, retail, and marketing experts) could also be beneficially conducted.

Potential initiatives for stakeholders with the goal of facilitating the competitiveness of cage-free systems of egg production in Asia, as suggested by the perceptions of egg producers in the present study, are presented below.

Suggested initiatives

- Conduct robust economic modeling to demonstrate the commercial feasibility of modern cage-free farms.
- Increase the competitiveness of cage-free systems by investigating and refining efficiencies and management practices.
- Build the commercial feasibility of cage-free farms through (1) hosting up-skilling activities for existing cage-free farmers (summits, training programs, peer networks), (2) applying science and technology to improve cage-free systems, (3) apply high-end business and marketing principles to grow the market for cage-free eggs (commercial buyers, consumers, and distribution channels) to increase demand.
- Build awareness in egg industries on the realities of efficiently, well run, large-scale commercial cage-free systems.
- Facilitate collaboration with egg producers and local governments to identify suitable land parcels on which to pilot cage-free growth/land parcel program.
- Partnerships with government and industry associations to offer training programs and industry showcases.
- Establish modern cage-free model farms that exhibit best practice and are demonstrable as economic models conducive to a profitable business.
- Apply technology and innovation to develop improved general on-farm management practices, including bird health, bird security, disease mitigation, feed distribution, flock sizes, and behavioral management.
- Apply science and technology to research and develop an improved feed conversion ratio in cage-free farms in the region.
- Increase knowledge and training for cage-free systems, for example by developing cage-free best practice management training programs and sponsor key stakeholders to attend, with a special focus on effective disease mitigation strategies/biosecurity and food safety.

TABLE 7 Comparison of the perceived benefits in improving animal welfare in a previous study with livestock leaders in Asia (9) in relation with Asian egg producers in the present study.

Rank*	Benefit by "importance" (9)*	Comparative benefit "top 10" findings in present study (2022)**	
		Cage	Cage-free
1	Productivity of the animals; Improve quality of meat or animal product <100%>	Increased productivity/yield	Product quality/price point
2	Reduce disease and injury and treatment costs <53%>	Reduce cost	Improved bird health
3	Avoid cruelty and reduce animal suffering <53%>		Improved animal welfare
4	Increased revenue/profit <47%>	Reduce cost Ease/convenience of management Land optimization Scalability General efficiency of resources	Low investment cost Wider market access/increasing demand/brand/differentiation Access to international markets/ keeping up with modern global practices/EU standards General cost saving
5	Human health/zoonosis; Protection of natural resources/ecosystem development <35%>	Hygiene of product Biosecurity/disease transmission	

"Rank" indicates a rank in importance across the countries from the findings in the previous study on the generalized benefits of addressing animal welfare in animal agriculture.
* <%> indicates the percentage of focus groups ($n = 17$) in which the listed benefit was presented by livestock leaders.
** <%> indicates the percentage of countries in which the benefit was presented as an important theme.

- Workshop solutions and sponsor research and development into addressing the challenges raised in this study, including financial obstacles, including both internally within a company and externally through investors, banks and government support or subsidies.
- Develop resource hubs on best practice management, biosecurity and disease prevention and treatment on cage-free farms, including up to date information on automation and science.

Limitations

This study represents an initial explorative study. For this reason, this study is foundational, and should be regarded as useful general information and a platform from which to continue more in-depth studies. While this study does not provide a definitive list of potential benefits and challenges in adopting cage-free systems, it does, however, provide initial insight into the benefits the participating egg producers see as possible and important.

A limitation of this study is the investigatory "wide-net" nature, which was designed to investigate an area that has scarcely been researched previously. There is also a lack of quantification around the strength of each item including, in this case, the reasons to operate the different systems, and each "barrier" and each "solution" identified. Further, the format of the methods meant an inability to further question producers in relation to meanings and details of their answers. Another unavoidable limitation was the need to translate all of the information twice. Furthermore, in some areas there is large variability between farm sizes (e.g., caged-farm size in the Philippines ranged from 15,000 to 900,000 birds). While the aim was to target producers from farms that are sizeable enough to be representative of the industry in each local area, some differences may be found in the operation of farms at varied sizes within this range.

While this study sets a useful foundation, it also provides some advice on conducting further quantitative and qualitative investigations in the region.

Conclusion

This study aimed to better understand the perceived barriers and potential benefits for the egg industry in considering the adoption of cage-free systems. It also investigated the possible solutions to the barriers. These barriers, benefits, and solutions are discussed, and result-advised applications are suggested. The findings suggest that a multi-faceted approach is needed to overcome the barriers that egg producers face in considering a move to cage-free systems, and in implementing solutions. The substantial list of solutions and support needed presented by producers in this study, represents vast opportunities to develop applications that may carry an increased likelihood of engagement with egg producers, and provide support in the way that support is needed.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the University of Queensland Human Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

Author contributions

MS conceptualized the project, created methodology, coordinated data collection, conducted analysis, and wrote the paper. KH conceptualized the project, created methodology, and contributed to writing the paper. QY, ML, and ZI contributed to methodology, conducted data collection, and edited the paper. AA, SI, RI, and JJ conducted data collection. EL conceptualized the project and edited the paper. JN initiated and conceptualized the study. All authors contributed to the article and approved the submitted version.

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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

References

1. Food and Agricultural Organisation. *FAOSTAT 'Livestock Primary' Data 2018*. (2020). Available online at: http://www.fao.org/faostat/en/#data/QL (accessed April 25, 2022).

2. Poultry Hub. *Chicken Egg (Layer) Industry*. Poultry Hub. (2020). Available online at: http://www.poultryhub.org/production/industry-structure-and-organisations/egg-industry (accessed November 6, 2020).

3. Yang L. *Caged-Laying Hens or Cage-Free Eggs: A Qualitative Study on Chinese Egg Producers' Views on Animal Welfare and Adopting Cage-Free Systems*, (MSc thesis), The University of Edinburgh, Edinburgh, United Kingdom (2020).

4. Clauer P. *History of the Chicken*. Pennsylvania State University. (2011). Available online at: https://extension.psu.edu/history-of-the-chicken (accessed July 22, 2021).

5. Grandin T. The effect of economic factors on the welfare of livestock and poultry. In: T Grandin, editor, *Improving Animal Welfare: A Practical Approach*, Oxfordshire: Cabi (2015). p. 1–14. doi: 10.1079/9781780644677.0000

6. Yu J, Wu J. The sustainability of agricultural development in China: the agriculture–environment nexus. *Sustainability*. (2018) 10:1776. doi: 10.3390/su10061776

7. Sinclair M, Phillips CJC. The cross-cultural importance of animal protection and other world social issues. *J Agri Environ Ethics.* (2017) 30:439–55. doi: 10.1007/s10806-017-9676-5

8. Li PJ. *Animal Welfare in China: Culture, Politics and Crisis*. Sydney: Sydney University Press (2021). doi: 10.2307/j.ctv1g13jzv

9. Sinclair M, Lee NYP, Hötzel MJ, de Luna MCT, Sharma A, Idris M, et al. International pereceptions of animals and their welfare. *Front Anim Sci.* (2022) 3:960379. doi: 10.3389/fanim.2022.960379

10. Sinclair M, Zhang Y, Descovich K, Phillips CJC. Farm animal welfare science in China-a bibliometric review of Chinese literature. *Animals.* (2020) 10:504. doi: 10.3390/ani10030540

11. Sinclair M, Zito S, Idrus Z, Yan W, Nhiem D, Lampang P, et al. Attitudes of stakeholders to animal welfare during slaughter and transport in SE and E Asia. *Anim Welfare.* (2017) 26:417–25. doi: 10.7120/09627286.26.4.417

12. Sinclair M, Fryer C, Phillips CJC. The benefits of improving animal welfare from the perspective of livestock stakeholders across Asia. *Animals.* (2019) 9:123. doi: 10.3390/ani9040123

13. Sinclair M, Phillips CJC. Key tenets of operational success in international animal welfare initiatives. *Animals.* (2018) 8:6. doi: 10.3390/ani8060092

14. Poultry World. *Companies Continuing to Meet Cage-Free Commitments*. Poultry World. (2021). Available online at: https://www.poultryworld.net/poultry/companies-continuing-to-meet-cage-free-commitments/ (accessed April 26, 2022).

15. Compassion in World Farming. *Egg Track 2021 Report*. (2022). Available online at: https://www.compassioninfoodbusiness.com/media/7447936/2021_eggtrack_report-final.pdf (accessed April 26, 2022).

16. Malone T, Lusk J. Putting the chicken before the egg price: an "ex post" analysis of california's battery cage ban. *J Agri Resour Econ.* (2016) 41:518–32.

17. Hannah J, Wilson J, Cox N, Cason J, Bourassa D, Musgrove M, et al. Comparison of shell bacteria from unwashed and washed table eggs harvested from caged laying hens and cage-free floor-housed laying hens. *Poult Sci.* (2011) 90:1586–93. doi: 10.3382/ps.2010-01115

18. Weary DM, Ventura BA, Von Keyserlingk MAG. Societal views and animal welfare science: understanding why the modified cage may fail and other stories. *Animals.* (2015) 10:309–17. doi: 10.1017/S1751731115001160

19. Gill P, Stewart K, Treasure E, Chadwick P. Methods of data collection in qualitative research: interviews and focus groups. *Br Dent J.* (2008) 204:291–5. doi: 10.1038/bdj.2008.192

20. QSR International. *Products*. Melbourne: Nvivo (2018). Available online at: http://www.qsrinternational.com/products_nvivo-mac.aspx (accessed July 1, 2021).

21. Appleby MC, Hogarth GS, Anderson JA, Hughes BO, Whittemore CT. Performance of a deep litter system for egg production. *Br Polutry Sci.* (1988) 29:735–51. doi: 10.1080/00071668808417102

22. Aladejebi OJ, Okojie LO, Afolami CA. Comparitive production efficiency of battery cage and deep litterl system in the drive towards sustainable poultry egg farming in Ogun State, Nigeria. *J Sustain Dev Africa.* (2014) 16:139–59.

23. Rajendran K, Mohanty S. Comparative economic analysis and constraints in egg production under cage vs. deep litter systems of rearing in India. *Int J Polutry Sci.* (2003) 2:153–8. doi: 10.3923/ijps.2003.153.158

24. Matthews WA, Sumner DA. Effects of housing system on the costs of commercial egg production. *Poult Sci.* (2015) 94:552–7. doi: 10.3382/ps/peu011

25. Ward J. From battery cages to barns: a cost-benefit analysis of a national standard for cage-free egg production. *Sch Publ Pol Capstones.* (2014) 34:1–14.

26. Schuck-Paim C, Negro-Calduch E, Alonso WJ. Laying hen mortality in different indoor housing systems: a meta-analysis of data from commercial farms in 16 countries. *Sci Rep.* (2021) 11:3052. doi: 10.1038/s41598-021-81868-3

27. McKinsey and Company's Retail and Consumer Goods. *Perspectives on Retail and Consumer Goods*. Chicago, IL: McKinsey & Company (2020).

28. Li PJ. *The Evolving Animal Rights and Welfare Debate in China: Political and Social Impact Analysis. Animals, Ethics and Trade. The Challenge of Animal Sentience*. (2006). p. 111–28. Available online at: https://www.wellbeingintlstudiesrepository.org/cgi/viewcontent.cgi?article=1054&context=acwp_awap (accessed April 25, 2022).

29. Thornton O. *Animal Welfare Around the World: Examining the Role the Economy Has on Animal Welfare*. Knoxville, TN: The University of Tennessee (2013).

30. Sinclair M, Lee NYP, Hötzel MJ de Luna MCT, Sharma A, Idris M, et al. Consumer attitudes towards egg production systems and hen welfare across the world. *Front Anim Sci.* (2022) 3:995430. doi: 10.3389/fanim.2022.995430

31. Hannah J, Wilson J, Cox N, Richardson L, Cason J, Bourassa D, et al. Horizontal transmission of Salmonella and Campylobacter among caged and cage-free laying hens. *Avian Dis.* (2011) 55:580–7. doi: 10.1637/9717-031511-Reg.1

32. Parisi MA, Northcutt JK, Smith DP, Steinberg EL, Dawson PL. Microbiological contamination of shell eggs produced in conventional and free-range housing systems. *Food Control.* (2015) 47:161–5. doi: 10.1016/j.foodcont.2014.06.038

33. Harper GC, Makatouni A. Consumer perception of organic food production and farm animal welfare. *Br Food J.* (2002) 104:287–99. doi: 10.1108/00070700210425723

34. World Population Review. (2022). China population: Live. Available online at: http://worldpopulationreview.com/countries/china-population/ (accessed July 1, 2022).

35. Bennett R. The value of farm animal welfare. *J Agri Econ.* (1995) 46:46–60. doi: 10.1111/j.1477-9552.1995.tb00751.x

36. Mench JA, Sumner DA, Rosen-Molina JT. Sustainability of egg production in the United States—the policy and market context. *Poult Sci.* (2011) 90:229–40. doi: 10.3382/ps.2010-00844

37. Sinclair M, Lee HP, Chen M, Li X, Mi J, Chen S, et al. Opportunities for the progression of farm animal welfare in China. *Front Anim Sci.* (2022) 3:89377. doi: 10.3389/fanim.2022.89377

38. Hartcher K, Jones B. The welfare of layer hens in cage and cage-free housing systems. *World's Poult Sci J.* (2017) 73:767–82. doi: 10.1017/S0043933917000812

39. Sinclair M, Idrus Z, van Nhiem D, Katawatin S, Todd B, Burns GL, et al. Motivations for industry stakeholders in China, Vietnam, Thailand and Malaysia to improve livestock welfare. *Animals.* (2019) 9:416. doi: 10.3390/ani9070416

40. Baker P, Dijk LV. *Hennovation*. Bristol: University of Bristol. (2021). Available online at: https://hennovation.eu (accessed July 22, 2021).

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Ackonc-AWA: A multi-species animal welfare assessment protocol for wild animals under human care to overcome the use of generic welfare checklists

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Introduction: Maintaining a high level of animal welfare is essential in zoos, sanctuaries and aquaria for ethical, legislative and functional reasons. Therefore, it is necessary to have welfare assessment protocols that can be incorporated into daily management programs. Currently, there are different approaches to assessing animal welfare in zoos. Those that can be applied to multiple species consist of checklists or qualitative assessments, with limitations, especially regarding the lack of guidance in the selection and interpretation of indicators. Validated protocols also exist, but they are for very few wild species. This study aimed to develop, test in the field, and describe an animal welfare assessment protocol for wild animals under human care, that can be applied to multiple species, intended to overcome the use of generic welfare checklists and offer an alternative to challenging and time consuming species-specific tools.

Methods: The development process consisted of the elaboration of a protocol, substantiated by published literature on zoo animal welfare and multidisciplinary focus group work, and its on-field feasibility test. This was performed on 14 species of different taxa housed in an Argentinian zoo. The protocol was structured in two forms: an initial form to serve as scan using various animal-based (ABM), resource-based (RBM), and management-based measurements (MBM), and a follow-up form using exclusively ABM. The protocol also included a user's manual with information about preliminary preparation, equipment required, steps from arrival until completion, and details on how to assess each indicator. The scoring method consisted in rating each indicator on a 3-point scale.

Results: 23 ABM, 19 RBM, and three MBM were tested and selected to integrate Ackonc-AWA, a multidimensional protocol covering the five animal welfare domains and applicable to multiple species.

Discussion: This protocol was entirely developed in Spanish and can be applied noninvasively and at a low cost, which constitute features of high relevance for Latin America. Further applications of the described welfare

assessment tool in other species and different institutional contexts will reinforce the validation of the proposed measurements and allow the systematic and routine evaluation of animal welfare in zoos.

KEYWORDS

animal-based measurements, animal welfare, assessment protocol, compassionate conservation, management-based measurements, resource-based measurements, zoo, animal welfare indicators

Introduction

Individual animal welfare and species welfare are critical obligations of zoos, sanctuaries, and aquaria (hereafter simplified as "zoo(s)"). Even the most ambitious conservation goals will not be adequate justification for keeping wild animals in captivity if zoos do not actively demonstrate high standards of animal welfare (1–4). The integration of animal welfare and wildlife conservation has been reflected in the emergence of new fields of study, such as compassionate conservation and conservation welfare. These multidisciplinary approaches attribute intrinsic value to some individual wild animals and support our moral obligation to consider their welfare, interacting with responsibilities to protect other aspects of nature, such as populations, species, ecosystems and biodiversity (5–8). Despite these similarities, there are differences in their ethical foundations, and pragmatism that have been deeply discussed in the literature [e.g., (8, 9)].

The past few decades have seen an increased interest in animal welfare among researchers and zoo staff. Zookeepers identify training in this area as relevant and important to their work (9) and the scientific community shows an increase in published research on animal welfare over time (10–12). In addition, there is a growing public concern for animal welfare and an ethical requirement to comply with international standards and national regulations on zoo animal welfare (4, 13).

According to the Single Public Registry of Wildlife Operators (14), in Argentina there are 16 officially registered institutions that house wild fauna, with numerous populations of diverse native and exotic species, maintained under different conditions of animal welfare, and with dissimilar realities in terms of human and financial resources. In addition to the interest of researchers and zoo staff, the active demands of public opinion and animal rights NGOs have led to official interventions to initiate conversion processes in many zoos, with animal welfare as the main driver. It has also led to an update of national and territorial regulations, establishing animal welfare as a priority by applying the highest welfare standards for individuals, through adequate facilities and management modalities in zootechnical, ethological, sanitary, and genetic terms (15).

Ensuring animal welfare requires knowledge, experience, and institutional commitment, as well as the deployment of comprehensive and robust animal welfare assessment tools, which can be implemented at two levels: institutional (examining policies, resources, programs, and practices) or individual (providing an assessment of animals and their environments) (3, 16). As animal welfare is a multidimensional field of study (17–19), welfare assessment should consider multiple criteria (20–22), with a holistic evidence-based approach (3). Therefore, most welfare assessments strategically include animal-based measurements (ABM) that address aspects of the actual welfare state of the animals in terms of their behavior, mental state, health, and physical condition. They also incorporate resource-based (RBM) and management-based measurements (MBM) that can be correlated to ABM and used to identify risks for animal welfare and causes of poor welfare, so as to implement improvement strategies (23).

The approach in the construction of protocols to assess animal welfare, their methods, and the way in which they should be evaluated or validated depend on the goals, which need to be clearly defined before starting the development process. Botreau et al. (20) identify three main models for assessing animal welfare according to the intended goals: descriptive, normative, and prescriptive. The descriptive model is used to depict a pre-existing situation that is stable and independent of any observation, thus providing the ability to characterize and compare observed situations. The normative model explains how things should be or how people should act, and aims to provide evaluation procedures to verify the appropriateness of collected information in relation to predefined rules. Finally, the prescriptive approach does not assume any pre-existing situation to be described; it aims to collect and organize relevant information to facilitate the formulation of recommendations to achieve a goal.

Currently, there are different tools to assess animal welfare in zoos. Those that can be applied to multiple species usually consist of extensive checklists with questions aimed at revealing what the conditions of the physical and social environment are like and provide insight into the welfare of an individual animal [e.g., (3, 16, 24)]. Some of them also consider and integrate life stages, in relation to species and individual differences

[e.g., (25)]. Although these protocols can be useful to easily improve animal welfare monitoring, they have some limitations, especially regarding the lack of guidance in the selection and interpretation of indicators, and thus, a non-tested reliability on applicants' criteria. Validated protocols also exist (21, 26–30), but they have been developed specifically for very few of the enormous variety of wild animal species that could require assessment (24).

This study aimed to develop, test in the field, and describe an animal welfare assessment protocol for wild animals under human care, which can be applied on a daily basis, noninvasively, and at a low cost, under the aforementioned prescriptive model. That is, first the current welfare status of the animal is assessed to understand the starting point and then its evolution is monitored by collecting information that allows the development of tailor-made recommendations and rapid decision making. Hence, it was intended that the protocol would be able to provide two types of assessment: comprehensive (whether initial diagnosis or in the face of important events, such as changes in the environment, group structure, and/or management) and regular (frequent monitoring to detect early deviations). Simultaneously, it aimed to obtain an intermediate solution between protocols that are easy to apply yet rely entirely on the judgment of the assessors, and validated but species-specific protocols that are useful only for assessing the species for which it was developed.

Materials and methods

Site

The protocol was tested at an Argentinean zoo, member of Asociación Latinoamericana de Parques Zoológicos y Acuarios (ALPZA) and World Association of Zoos and Aquariums (WAZA), which was in the process of transformation and restructuring. The protocol was applied and tested between October and December 2017.

Elaboration of the protocol

The protocol was given the name Ackonc-AWA, which combines the purpose of conducting animal welfare assessments (AWA) with the role of the individuals involved in the observation and data collection process (hereafter, sentinels), given that the phonetics of the name reflects the native Andean word "ackoncahua" which is translated as "sentinel".

The conceptual animal welfare framework adopted to create Ackonc-AWA protocol was the Five Domains Model (31), with a joint approach between the behavioral domain and the mental domain. Based on a literature review through research databases (PubMed and Google Scholar), with date restriction from January 2008 to July 2017, in English and Spanish, a selection of scientifically supported indicators previously used in welfare assessment protocols applied to farm, laboratory and zoo animals was obtained. Some of these indicators and their references were modified to adapt them to the characteristics of the zoo, to the variety and characteristics of species to be evaluated, and taking into account previous experiences of the researchers on animal welfare assessment in zoos. Interviews and meetings with personnel from different areas of the zoo were conducted. During the interviews, questions related to animal welfare were asked (e.g., When you observe the animals under your care, what do you look at? How do you notice if there is any discomfort, pain or something wrong with them?). Their responses were taken into account when selecting, eliminating, or adapting certain indicators in the protocol.

The principle of feasibility was taken into account for the selection of the welfare indicators (32, 33). The researchers also considered the need for the institution's own staff to be able to collect the data easily, subject to adequate training and performance evaluation. Thus, all measurements involving physical invasion or restraint of the animals, and indicators that require further laboratory analysis (e.g., metabolic profiling), were excluded. For this test, all the animal welfare assessments were performed hands-off, by remote observations at a distance.

In addition, two meetings were held with eleven representatives of different areas of the zoo (Veterinary, Nutrition, Biology, Behavior, Animal Care, Animal Welfare Management and Planning) to submit their input to a multi-disciplinary discussion in a focus group in order to select agreed upon items for assessment, as a way to provide content validity (13).

Once the first selection of the indicators to be assessed had been made, two types of forms were developed: an initial form and a follow-up form. The initial form consisted on 45 indicators (23 ABM, 19 RBM, and three MBM) (Table 1), which was meant to be carried out the first time an animal is assessed, and then on a semi-annual basis, or in the face of important changes in the environment, group structure and/or management of the animal under study. At this first step, the RBM and MBM were exhaustively considered together with ABM, to detect risk factors of poor welfare, even before the occurrence of identifiable manifestations by means of ABM. The follow-up form consisted exclusively of ABM (23 indicators) to facilitate the data collection process and reduce the time required to carry out the observations, and was intended to be applied daily or weekly. The frequency of use of the follow-up form can be adjusted according to need. As a starting point, the researchers suggest a weekly application. However, a higher frequency (i.e., daily) could be used for continuous monitoring of a newly moved animal or changes in group composition, management or enclosure characteristics to detect early alterations in ABMs that reflect a deterioration in welfare.

TABLE 1 List of indicators selected to test on-field reliability and feasibility, and sentinels assigned according to their availability, area of daily performance and experience.

	Sentinels assigned to the on-field feasibility and reliability test of each indicator						
	External	Zoo staff (departments)					
	Researchers	V	N	Bi	Be	AC	AWMP
Nutrition domain							
Body condition score (ABM)	Yes	Yes	Yes	No	No	Yes	Yes
Food intake (ABM)	Yes	Yes	Yes	No	No	Yes	Yes
Food availability (RBM)	Yes	No	Yes	Yes	No	Yes	Yes
Nutritional quality and safety of food (RBM)	Yes	No	Yes	No	No	No	Yes
Macroscopic condition of food (RBM)	Yes	No	Yes	No	No	No	Yes
Food presentation (RBM)	Yes	No	Yes	No	Yes	Yes	Yes
Water intake (ABM)	Yes	No	Yes	No	No	Yes	Yes
Availability of water (RBM)	Yes	No	Yes	Yes	No	Yes	Yes
Macroscopic quality of water (RBM)	Yes	No	Yes	No	No	Yes	Yes
Presentation of water (RBM)	Yes	No	Yes	Yes	Yes	Yes	Yes
Environment domain							
Substrate (RBM)	Yes	No	No	Yes	Yes	Yes	Yes
Temperature/humidity/ventilation (RBM)	Yes	Yes	No	No	No	Yes	Yes
Lighting (RBM)	Yes	Yes	No	Yes	No	Yes	Yes
Enclosure maintenance (RBM)	Yes	No	No	No	No	Yes	Yes
Enclosure hygiene (RBM)	Yes	No	No	No	No	Yes	Yes
Enclosure dimensions (RBM)	Yes	No	No	Yes	Yes	Yes	Yes
Environmental complexity (RBM)	Yes	No	No	Yes	Yes	Yes	Yes
Surrounding enclosures (RBM)	Yes	No	No	No	Yes	Yes	Yes
Shelter availability (RBM)	Yes	No	No	No	Yes	Yes	Yes
Public (RBM)	Yes	No	No	No	Yes	Yes	Yes
Group composition (RBM)	Yes	No	No	Yes	No	No	Yes
Environmental choice and control opportunities (RBM)	Yes	No	No	No	No	Yes	Yes
Management choice and control opportunities (MBM)	Yes	No	No	No	Yes	Yes	Yes
Environmental enrichment (MBM)	Yes	No	No	No	Yes	Yes	Yes
Training procedures (MBM)	Yes	No	No	No	Yes	Yes	Yes
Health domain							
Defecation behavior (ABM)	Yes	Yes	No	No	No	Yes	Yes
Stool score (ABM)	Yes	Yes	No	No	No	Yes	Yes
Micturition behavior (ABM)	Yes	Yes	No	No	No	Yes	Yes
Urine appearence (ABM)	Yes	Yes	No	No	No	Yes	Yes
Coat/feathers/tegument (ABM)	Yes	Yes	No	No	No	Yes	Yes
Lesions/injuries (ABM)	Yes	Yes	No	No	No	Yes	Yes
Hooves/claws/teeth (ABM)	Yes	Yes	No	No	No	Yes	Yes
Locomotion (ABM)	Yes	Yes	No	No	No	Yes	Yes
Sleep/wakefulness (ABM)	Yes	Yes	No	Yes	No	Yes	Yes
Signs of illness (ABM)	Yes	Yes	No	No	No	Yes	Yes
Behavior and affective states domain							
Reaction to strangers (ABM)	Yes	No	No	No	Yes	Yes	Yes
Interaction with zookeepers (ABM)	Yes	No	No	No	Yes	Yes	Yes
Exploration (ABM)	Yes	No	No	No	Yes	Yes	Yes
Social, affiliative and maternal-filial behavior (ABM)	Yes	No	No	No	Yes	Yes	Yes

(Continued)

TABLE 1 (Continued)

	Sentinels assigned to the on-field feasibility and reliability test of each indicator						
	External	Zoo staff (departments)					
	Researchers	V	N	Bi	Be	AC	AWMP
Reproductive behavior (ABM)	Yes	No	No	Yes	Yes	Yes	Yes
Agonistic behavior (ABM)	Yes	No	No	No	Yes	Yes	Yes
Use of environmental enrichment (ABM)	Yes	No	No	No	Yes	Yes	Yes
Stereotypic behavior (ABM)	Yes	No	No	No	Yes	Yes	Yes
Behavioral diversity (ABM)	Yes	No	No	Yes	Yes	Yes	Yes
Space use (ABM)	Yes	No	No	No	Yes	Yes	Yes

V, Veterinary; N, Nutrition; Bi, Biology; Be, Behavior; AC, Animal Care; AWMP, Animal Welfare Management and Planning; ABM, Animal-based measurement; RBM, Resources-based measurement; MBM, Management-based measurements.

Additionally, a user's manual was written with instructions on the method used to assess and score each indicator. Both the indicators selected and the instructions for their assessment were the same for the different species and individuals included in the pilot test, although changes and clarifications were made in the user's manual to adapt them to the differential characteristics of each taxon.

Before beginning the assessment, sentinels had to be familiar with the following information about the species to be assessed: biological and behavioral features (including specie's ethogram); housing and handling requirements recommended by international associations; nutritional information (diet received by the animal or group being evaluated) and both routine and scheduled activities (e.g., feeding time, enclosure cleaning, training sessions, environmental enrichment, animal rotation and other interfering activities planned for the day of the assessment, such as capture for veterinary examination or transfer to another enclosure). Likewise, sentinels should have a layout/map and information about the location and dimensions of the enclosure.

Every effort should be made to minimize the impact of the presence of the sentinels on the behavior of the animal under study. Sentinels should remain out of sight and avoid any kind of interaction with the observed animal during the data collection to minimize the impact of his or her presence on the behavior of the animal under study (e.g., choosing an observation point to allow the sentinel to be as hidden as possible or remaining as long as necessary without interacting with the animal until it withdrew its attention from the sentinel's presence).

Indicators were rated on a 3-point scale (A—normal/no observable welfare risk; B—mild deviation/welfare risk; C—severe deviation/welfare risk). For indicators that could be rated in several contexts (e.g., animals that have access to different enclosures at different times), rating was made according to the context that represented a higher level of animal welfare compromise. When any indicator was rated "B" or "C", the sentinel provided additional information about this on the "Notes" column.

On-field feasibility test

Animals

The selection of the species and individuals on which the protocol was tested was based on the following inclusion criteria: 1—prospective permanence of the animals in the zoo: longer than 2 years; 2—easy identification: phenotypic characteristics or features that made it possible to individualize the animals housed in groups; and 3—include species from different taxonomic categories to test the ability of Ackonc-AWA protocol to be applied for different taxa. As a result, 14 individuals (ten mammals, two birds, and two reptiles) from different orders and families were selected (Table 2). Ackonc-AWA was also tested on one group of 12 capybara (*Hydrochoerus hydrochaeris*), to explore the potential usefulness of the protocol for group assessment, with proper modifications or adaptations.

Sentinels

Sentinels assigned to observe, record and score the indicators on site were selected from the different areas involved in animal management and care, based on interviews, in search of those who met a combination of experience, training, predisposition and observation skills. Their election was also agreed with representatives of the institution in order to avoid hindering or disrupting daily activities. Hence, the team of sentinels consisted of a group of three external veterinarians experienced in animal welfare assessments (the first three authors of this work, hereafter the researchers) and a group of nine zoo staff members with no prior experience in animal welfare assessments, belonging to different departments [one

TABLE 2 Information about the animals on which the Ackonc-AWA protocol was tested.

	Family	Species	Gender	Age (Years)	Level of assessment
Order mammals					
Primates	Hominidae	*Pan Troglodytes*	Male	11	Individual
		Pongo spp.	Female	31	
Carnivora	Canidae	*Chrysocyon brachyurus*	Female	16	
	Felidae	*Panthera tigris tigris*	Male	11	
	Otariidae	*Otaria flavescens*	Female	10	
Pilosa	Myrmecophagidae	*Myrmecophaga tridactyla*	Female	12	
Proboscidea	Elephantidae	*Elephas maximus*	Female	50	
Perissodactyla	Tapiridae	*Tapirus terrestris*	Male	10	
Artiodactyla	Camelidae	*Vicugna vicugna*	Male	13	
Rodentia	Caviidae	*Hydrochoerus hydrochaeris*	6 females 5 males 1 Unknown	11 adults 1 young	Individual and Group
Order birds					
Cathartiformes	Cathartidae	*Vultur gryphus*	Male	10	Individual
Psittaciformes	Psittacidae	*Anodorhynchus hyacinthinus*	Female	27	
Order reptiles					
Testudines	Chelidae	*Acanthochelys spixii*	Female	4	Individual
Squamata	Teiidae	*Salvator rufescens*	Male	8	

from Veterinary, one from Nutrition, one from Biology, two from Behavior, two from Animal Care (zookeepers) and two from Animal Welfare Management and Planning (AWMP)].

All inexperienced sentinels received a 4 h theoretical and practical training on animal welfare assessment in general and on the use of the protocol in particular, designed and delivered by the researchers. A virtual library was also created with ethograms and information on each of the 14 species' nutritional, physiological, environmental and behavioral needs, selected by the researchers from books, husbandry manuals and peer-reviewed scientific publications. All sentinels were given access to this virtual library and were instructed to read the documents selected for the corresponding species before beginning the on-field feasibility test.

The researchers and the zoo staff from the AWMP Department were exclusively dedicated to this task, so they evaluated the entire protocol (all indicators). On the other hand, the rest of the sentinels were assigned a different number of indicators to score, since they had different availability to collaborate with this research (Table 1). For the latter group, indicators would be scored during the zoo routine schedule and with minimum interference to the daily management and procedures. Likewise, the assignment of the indicators to be rated was made considering their area of daily performance and previous experience. For instance, health-related indicators were assigned to the zoo veterinarian, and nutrition-related indicators to the nutrition expert.

Test-retest reliability

Three sentinels were assigned the assessment of the same animal at two different time points. Test-retest agreement rate was corrected for chance by kappa statistics (34). Inter-observer reliability could not be assessed due to the limited availability of zoo staff involved in this pilot test. The statistical processing of the data was carried out using the software Infostat® (35) and VassarStats: Website for Statistical Computation (36).

Feasibility

Completeness of the forms

For the animal welfare assessment to be comprehensive, all indicators in Ackonc-AWA must be completed, except for those that do not apply to a given species, due to its particular nature (e.g., water consumption in underwater species) or under specific situations (courtship behavior outside the reproductive season). In such cases, sentinels were instructed to use the legend "does not apply" to differentiate them from those that could be left blank due to other reasons (e.g., lack of time, impossibility of taking the measurement, not provided access/information).

The average completeness of the forms was determined by averaging the degree of completeness achieved by all sentinels for all species. In addition, a ranking of the indicators most often left blank was made by counting the number of times that each indicator was not evaluated when it should have, in relation to the total of forms (both initial and follow-up) across species and sentinel groups.

TABLE 3 Test-retest reliability calculated for the on-field test of Ackonc-AWA protocol.

	Cohen's kappa	SE	CI95%	Proportion of agreement	*n*
Sentinels					
1	0.7391	0.0798	(0.5827; 0.8955)	0.8421	57
2	0.7863	0.0821	(0.6255; 0.9471)	0.8846	52
3	0.7997	0.0623	(0.6777; 0.9217)	0.8696	69
Overall pondered	0.7763			0.8652	
Indicators					
ABM	0.7574	0.0759	(0.6086; 0.9062)	0.8816	76
MBM	0.6774	0.2040	(0.2775; 1)	0.8000	10
RBM	0.7877	0.057	(0.6761; 0.8993)	0.8636	88
Overall pondered	0.7681			0.8678	

Degree of difficulty represented by the observation and recording process

Ackonc-AWA protocol was designed so that the observations and completion of the forms can be done by the zookeepers, combining this activity with their other responsibilities. Therefore, it was important to determine the degree of difficulty perceived by the staff in applying the chosen indicators. For this purpose, after completing the Ackonc-AWA forms, each sentinel was asked to assign a degree of difficulty to fill out each form between 1 and 10, with 1 being the minimum and 10 the maximum. At the bottom of each form, the sentinels had to specify which indicator was found as the most difficult to evaluate. With these responses, the indicators were rated for their level of difficulty, from the most often reported to the least often reported. The results were analyzed by averaging the degree of difficulty assigned for all species and sentinels, differentiating between initial and follow-up assessment forms.

Time required to complete the forms

It was intended that the Ackonc-AWA protocol require <2 h per individual or group for data collection since long application protocols have more difficulties to be used regularly in zoological institutions, especially in those lacking resources or exclusive personnel for this purpose, a very frequent situation in Latin America. The average time (in minutes) required to complete the two welfare assessment protocol forms was recorded for all species and sentinels, differentiating between initial and follow-up assessment forms.

Ethical review of the project was requested to the Institutional Committee for the Care and Use of Laboratory Animals (CICUAL) of the Faculty of Veterinary Sciences of the University of Buenos Aires, and a review exemption was granted given the observational nature of the project.The study focused on the non-invasive/intrusive assessment of animal welfare, so no interventions of any kind were carried out on the animals. There were no potential adverse effects, nor foreseeable risks or hazards associated with this project, with regards to animal, plant and/or human wellbeing. The participation of zoo staff in this study was completely voluntary and under written informed consent. The survey responses were strictly confidential and data from this research was reported only in the aggregate. The information was coded and remains confidential.

Results

On-field feasibility test

Test-retest reliability

The mean intra-observer proportion of agreement was 0.8652 among the sentinels and 0.8678 among indicators (ABM, RBM, and MBM). The mean observed Kappa was 0.7763 among the sentinels and 0.7681 among indicators, which on the Landis and Koch (34) scale is substantial agreement (Table 3). Although Cohen's test ruled out a random component, more trials are needed to increase the statistical power of the test.

Feasibility

Completeness of the forms

The average completeness for the initial form was 86.21% whereas for the follow-up form it was 79.07%. The top ten indicators most often left blank were part of both assessment forms and were therefore analyzed together. No indicators were left blank over 50% of the times. Only Water intake was left blank over 40% of the times (56 times; 42.10%). Three indicators were left blank between 40 and 30% of times: Micturition behavior (44 times; 33.08%), Defecation behavior (42 times; 31.58%), and Use of environmental enrichment (42 times; 31.58%); two indicators were left blank between 30 and 20% of times: Social behavior (37 times; 27.82%) and Reproductive behavior (30 times; 22.56%); and four indicators were left blank between 20 and 10% of times: Hooves/claws/teeth (24 times; 18.04%), Agonistic behavior (20 times; 15.04%), Food intake (17 times; 12.78%) and Behavioral

TABLE 4 Time in minutes (mean +/− SD) required to complete initial and follow-up welfare assessment forms of Ackonc-AWA protocol.

Species	Initial form		Follow-up form	
	Mean	SD	Mean	SD
Pan Troglodytes	56.4286	28.9704	86.2500	22.5000
Pongo spp.	46.0000	29.6648	40.0000	0.0000
Chrysocyon brachyurus	47.8571	35.1019	31.2500	6.2915
Panthera tigris tigris	60.0000	46.9042	40.0000	28.2843
Otaria flavescens	66.6667	37.7712	37.5000	9.5743
Myrmecophaga tridactyla	29.0000	20.7364	67.5000	74.2462
Elephas maximus	46.0000	25.8360	75.0000	32.7872
Tapirus terrestris	55.0000	27.3861	87.5000	12.5831
Vicugna vicugna	30.0000	7.0711	50.0000	29.4392
Hydrochoerus hydrochaeris	63.2500	21.1213	58.2222	15.8096
Vultur gryphus	90.0000	42.4264	63.7500	25.6174
Anodorhynchus hyacinthinus	36.6667	23.5938	61.7500	13.3760
Acanthochelys spixii	41.6000	45.8290	43.7500	9.4648
Salvator rufescens	50.0000	18.7083	74.0000	43.6119
Total	51.3192	29.3658	58.3194	23.1132

diversity (16 times; 12.03%). The rest of the indicators included in the protocol were left blank <10% of the time.

Degree of difficulty represented by the observation and recording process

The mean reported difficulty across species and sentinels was 4.79 +/− 1.13 for the initial form and 5.20 +/− 1.51 for the follow-up form. Analysis of sentinel responses showed that the indicator most frequently reported as difficult to assess was Behavioral diversity (54 times; 40.60%) followed by Defecation behavior (31 times; 23.31%), Micturition behavior (29 times; 21.80%), Hooves/claws/teeth (13 times; 9.77%), Water intake (12 times; 9.02%) and Food intake (11 times; 8.27%).

Time required to complete the forms

The average time across species and sentinels required to complete the initial form was 51.32 min. +/− 29.36 min. Completion of the follow-up form took an average of 58.32 min +/− 23.11 min. Table 4 shows the amount of time (in minutes) required to complete initial and follow-up welfare assessment forms of Ackonc-AWA protocol for each of the species included in the study. Activity budget sheets were later added to the protocol (see below).

Structure of Ackonc-AWA and application guidelines/criteria

In the face of on-field feasibility results, some changes were implemented for the assessment of the indicator "Behavioral diversity" within the Behavioral and mental domain by the introduction of activity budget sheets of 20 min each, on three (or four, when possible) different time slots (see Supplementary Table 1). After sentinels complete the activity budgets sheets, a trained analyst (external or personnel of the institution) should evaluate the data and assign the appropriate score (A, B or C) for the indicators "Behavioral diversity" and "Space use".

No changes were made for the indicators included in the Nutritional, Environmental and Health domains. As a result, a total of 45 indicators (23 ABM, 19 RBM, and three MBM) were selected to integrate Ackonc-AWA, covering the five animal welfare domains.

Nutritional domain

Three ABM (Body condition score, Food intake, and Water intake) and seven RBM (Food availability, Nutritional quality and food safety, Macroscopic condition of food, Food presentation, Availability of water, Macroscopic quality of water, and Presentation of water) were selected to assess the nutritional domain. Table 5 summarizes the methods, references, and scoring system required for this purpose.

Environmental domain

Twelve RBM (Substrate, Temperature/humidity/ventilation, Lighting, Enclosure maintenance, Enclosure hygiene, Enclosure dimensions, Environmental complexity, Surrounding enclosures, Shelter availability, Public, Group composition, Environmental choice, and Control opportunities) and the three MBM (Management choice and control opportunities, Environmental enrichment, and Training procedures) were adopted. Table 6 summarizes the most relevant information provided in the user's manual for assessing environmental domain. To this end, sentinels had to be able to access and consider all areas destined to the animal (e.g., exhibitors, sleeping quarters, pens, handling areas, etc) to rate each indicator according to the sector(s) that imply a greater compromise to the welfare of the animal (or group).

Health domain

Ten ABM (Defecation behavior, Stool score, Micturition behavior, Urine appearance, Coat/feathers/tegument, Lesions/injuries, Hooves/claws/teeth, Locomotion, Sleep/wakefulness, and Signs of illness) were selected to assess the health domain. Table 7 summarizes the most relevant information provided in the user's manual for assessing health domain.

Given the multispecies purpose of the Ackonc-AWA protocol, it is important to note that for some species (e.g., reptiles, birds) it may be necessary to score the indicators

TABLE 5 Summary of the most relevant information that is provided in the user's manual for assessing nutritional domain.

Indicator	Method	Reference	Scoring
Body condition score (ABM)	It shall be assessed visually. Only when there is no risk for humans or animal's welfare, it may also be assessed by palpation. Use a standardized 5-point scale scientifically validated for the species under study.	Does the animal have a body condition appropriate to their species, age, sex and physiological state?	A: 3, B: 2 o 4, C: 1 o 5. In case B or C, clarify in "observations" to which BCS it corresponds.
Food intake (ABM)	Observe the eating behavior and the daily amount of food consumed.	Is the feed intake adequate for the animal according to their age, sex, physiological state and health condition?	A: normal appetite. B: hyporexia, pica, trichophagia, coprophagia (In some species coprophagia is not pathological). C: anorexia, polyphagia or any type of disturbance that is not allowing adequate food intake (even if appetite is not affected).
Food availability (RBM)	Observe the time at which food is offered in the indoor and outdoor enclosures (features to consider: number, competition for access, location and height, cleanliness and maintenance condition of feeders or feeding zone).	Is the food available and sufficient considering age, sex, physiological state and health condition of the animal?	A: all the features are adequate. B: only one of the features is not adequate, but it does not prevent access to the food. C: the food is not accessable and/or two or more features are not adequate.
Nutritional quality and safety of food (RBM)	Request information from the nutrition department. If possible, send food samples for analysis. Relevant literature should be used to obtain information on the reference values and analyses required for the species under study.	Is the diet adequate in nutrients (according to the species, age, physiological and health status) and are the ingredients safe and secure (free of contaminants and toxins, cold chain mantained)?	A: the diet is adequate, safe and secure. C: either nutrient profile or food safety criteria is not adequate.
Macroscopic condition of food (RBM)	Observe the food offered to the animal (alterations to consider: bruises, insects, mold, rotting, fruit ripening, fecal matter mixed with the food).	Is the food offered to the animal in good condition?	A: no alterations are observed. B: only one food or portion have only one of the mentioned alterations. C: one or more foods or portions have two or more of the mentioned alterations.
Food presentation (RBM)	Observe and compare the way in which the food is presented in the zoo with how it is found in the evolutionary environments of the species (features to consider: frequency, portion size, timing, texture, consistency, temperature and location).	Does the presentation of the food respect the way the species feeds in the wild?	A: all features to be considered are adequate. B: only one of the features is not adequate, but it does not impede the ingestion of food. C: two or more features are inadequate
Water intake (ABM)	Observe the drinking behavior and the daily amount of water consumed.	Does water consumption match the animal's requirements?	A: normal intake. B: slight increase or decrease in water intake unrelated to weather conditions. C: significant increase or decrease in water intake unrelated to weather conditions and/or difficulty in swallowing or ingesting water.
Availability of water (RBM)	Observe the water troughs and other water sources in the indoor and outdoor enclosures (features to consider: number, competition for access, location and height, cleanliness and maintenance).	Is the animal provided with sufficient and accessible water at all times?	A: all features to be considered are respected. B: only one of the features to be considered is not respected, but it does not prevent access to water. C: water is not accessible and/or two or more features to be considered are not respected.
Macroscopic quality of water (RBM)	Observe the water offered to the animal (features to consider: color, odor, presence of food debris and other visible particles, greenery)	Is the water offered to the animal in good condition?	A: all features to be considered are adequate. B: only one of the features to be considered is not adequate, but it does not prevent the ingestion of water. C: two or more features to be considered are not adequate.
Presentation of water (RBM)	Observe and compare the way in which water is presented in the zoo with how it is found in the evolutionary environments of the species and their drinking behavior.	Does the presentation of water respect the way it is found in the wild and accordingly with the species drinking behavior?	**A:** the presentation of water respects the way the species drinks in the wild. **B:** the presentation of water partially respects the way the species drinks in the wild (if the species has more than one way of drinking water, its presentation does not allow to express at least one of them) **C:** the presentation of water does not respect the way the species drinks in the wild.

ABM, Animal-based measurement; RBM, Resources-based measurement.

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TABLE 6 Summary of the information provided in the user's manual for assessing environmental domain.

Indicator	Method	Reference	Scoring
Substrate (RBM)	Observe the substrate of the enclosure and compare it with the typical natural environment of the species (features to consider: level of compaction, texture, hardness and temperature of the material, undulations and unevenness). If available, check the reference substrate requirements for the species in the husbandry manual.	Is the substrate suitable for the animal to rest comfortably and exhibit species-specific behaviors?	A: the substrate is suitable for the animal to rest comfortably and deploy species-specific behaviors. B: the substrate is inappropriate for the animal to rest comfortably or may prevent the manifestation of any species-specific behaviors. C: the substrate is inappropriate for the animal to rest comfortably and/or could prevent the manifestation of several species-specific behaviors.
Temperature/humidity/ventilation (RBM)	Observe the conditions offered in the enclosure and compare them with the climatic characteristics of the ancestral environment of the species (features to consider: sources of heat or cold, shade and sun, and bathing facilities (e.g., water, mud or other). If the enclosure has a controlled system for temperature, humidity and ventilation, or if you have a device to measure these parameters, check and record the values. If available, check the reference temperature, humidity and ventilation requirements for the species in the husbandry manual.	Are the enclosure conditions adequate to allow the animal to maintain thermal comfort?	A: the enclosure presents conditions that allow maintaining an adequate thermal comfort in all its aspects. B: one of the aspects of the enclosure is deficient to maintain adequate thermal comfort without threatening the life of the animal. C: two or more of the aspects of the enclosure are deficient to maintain adequate thermal comfort, or only one aspect is deficient in a way that puts the animal's life at risk.
Lighting (RBM)	Observe the lighting of the enclosure and compare it with the typical natural environment of the species. If available, check the reference lighting requirements for the species in the husbandry manual.	Does the lighting in the enclosure respect the circadian cycle, the number of hours of light/darkness characteristic of the natural environment of the species and does it not affect or hinder vision or generate somatic disorders? Is the amount of sunlight entering the enclosure adequate according to the characteristics of the natural environment of the species?	A: natural and artificial lighting is suitable for the species. B: one of the components to be considered is not appropriate, without putting the animal's life at risk. C: two or more of the components to be considered are deficient, or only one is deficient but puts the animal's life at risk.
Enclosure maintenance (RBM)	Observe the maintenance conditions of the enclosure (features to consider: defects in the structure of the enclosure that may cause damage to the animals, poisonous plants within reach, exposure to electrical appliances or poorly protected electrical outlets, vegetation that could fall and cause damage, entry of disease-carrying animals or pests such as rodents).	Does the condition in which the enclosure is maintained pose no risk to the health and welfare of the animal or third parties?	A: the enclosure is in good maintenance conditions. B: there are some defects in the maintenance of the enclosure, which do not directly endanger the lives of animals or people. C: there are many defects in the maintenance of the enclosure and/or the defective feature(s) put the life of animals or people at direct risk.

(Continued)

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TABLE 6 (Continued)

Indicator	Method	Reference	Scoring
Enclosure hygiene (RBM)	Observe the hygiene of the enclosure (features to consider: spoiled food, stagnant water, accumulation of feces and urine and dead animals). Consider that excess hygiene can also be detrimental (inadequate for the species or in higher concentration than recommended or with higher frequency than recommended). If available, check the recommended chemical types, concentration and frequency for the species (husbandry manual).	Is the enclosure maintained in adequate hygienic conditions? Are the chemicals used adequate in type and concentration? Is the frequency of cleaning adequate?	A: The enclosure is in good hygienic conditions and the cleaning routine is adequate for the species. B: there are some defects in the hygiene of the enclosure, which do not put the health of animals or people at direct risk. C: there are many defects in the hygiene of the enclosure and/or the defective feature(s) puts the health of animals or people at direct risk.
Enclosure dimensions (RBM)	Request the enclosure outline and verify that the declared dimensions match the actual dimensions. Take the necessary measurements and record the dimensions of the enclosure. When answering the reference question consider that the animal should be able to express the full repertoire of locomotor movements of their species, including running, climbing, flying or swimming at speed. If more than one individual is housed in the same enclosure, consider the number of animals per surface area. If available, check the reference requirements for the species (husbandry manual).	Do the dimensions of the enclosure allow the animal to move freely? Do they comply with the minimum space requirements stated in the husbandry manuals per individual?	A: the dimensions comply with existing recommendations and are adequate for the animal to move freely and express the full locomotor repertoire of its species. B: the dimensions allow the animal to move freely but hinder the expression of the full locomotor repertoire of its species and are below those recommended. C: dimensions do not allow the animal to move freely and/or impede the expression of the full locomotor repertoire of its species and are below those recommended.
Environmental complexity (RBM)	Observe the disposition of different areas and elements within the enclosure. Consider feeding and elimination zones, characteristics of the environment, land/water/air space ratio, implements for the vertical use of space. For an accurate evaluation of welfare it is essential to distinguish it from environmental enrichment.	Does the design of the enclosure allow for species-specific behaviors as well as differential use of each part of the space?	A: the design of the enclosure allows for differential use of each part of the space as well as the occurrence of all species-specific behaviors. B: the design of the enclosure allows differential use of each part of the space as well as the occurrence of most species-specific behaviors. C: the enclosure design does not allow differential use of each part of the space and/or prevents the occurrence of several of the species-specific behaviors.
Surrounding enclosures (RBM)	Observe the surrounding enclosures (features to consider: presence of visual barriers, pray, predators or competitors housed in adjacent enclosures and distance between enclosures).	Does the housing layout and design minimize stressful situations with animals in adjacent enclosures or loose animals?	A: the layout and design of the housing are adequate to minimize stressful situations with animals in adjacent enclosures or loose animals. B: only one of the features to be considered is deficient. C: two or more of the features to be considered are deficient.
Shelter availability (RBM)	Observe the existence, availability and adequacy of shelters for various weather conditions.	Do the animals have shelters to protect them from adverse weather conditions?	A: shelters provide full protection from inclement weather. B: shelters provide partial protection from inclement weather. C: shelters do not provide protection from inclement weather or there is no shelter or repair.

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TABLE 6 (Continued)

Indicator	Method	Reference	Scoring
Public (RBM)	Observe the possibility of hiding from the public (features to consider: visual barriers, impediments for direct contact; first, second and third level barriers; free access to confinement areas).	Does the housing layout and design minimize stressful situations for the animal generated by the public?	A: the layout and design of the housing are adequate to minimize stressful situations with humans. B: only one of the features to be considered is deficient. C: two or more of the features to be considered are deficient.
Group composition (RBM)	Observe group composition, ALWAYS record in "observations": number of adults (clarifying the sex of each one), juveniles (sex) and young, number of species and individuals in the same enclosure. If the enclosure is shared with another species, consider if this association is adequate for the species you are working with. (Features to consider:gregarious/solitary, number of individuals, proportion of males/females and offspring)	Is the group composition representative of the species?	A: the composition of the group is representative of the species in all features. B: the gregarious/solitary condition of the species is respected but one or more of the other features to be considered is deficient. C: the gregarious/solitary condition of the species is not respected and/or two or more of the other features to be considered are deficient.
Environmental choice and control opportunities (RBM)	Examine the enclosure and assess whether it offers the animals opportunities for control and choice. Consider: opportunities for choice of display or concealment, shade or sun, heat or cold, companionship or solitude, need to alternate exit to the main exhibit, access to the main exhibit during peak periods of the day—species with nocturnal or crepuscular habits, isolation from stressors derived from cleaning, maintenance and repair maneuvers.	Does the enclosure design allow the animal to choose where to be or what to do 24 h a day?	A: the design of the enclosure allows the animal to choose where to be or what to do, in all its aspects, during 24 h of the day. B: the enclosure design allows the animal to choose where to be or what to do, in various aspects, during at least the most active period of the day for the species. C: the enclosure design allows the animal to choose where to be or what to do in few or none of its aspects and/or opportunities for choice and control are present only during the period of the day of least activity for the species.
Management choice and control opportunities (MBM)	Interview staff and assess whether the management offers animals opportunities for control and choice. Consider all the aspects mentioned in "Environmental choice and control opportunities"	Does management allow the animal to choose where to be or what to do 24 h a day?	A: management allows the animal to choose where to be or what to do, in all its aspects, 24 h a day. B: management allows the animal to choose where to be or what to do, in several of its aspects, during at least the most active period of the day for the species. C: management allows the animal to choose where to be or what to do, in few or none of its aspects, and/or opportunities for choice and control are present only during the period of the day of least activity for the species.
Environmental enrichment (MBM)	Interview staff, check documentary records and verify the implementation of an appropriate and comprehensive environmental enrichment (EE) program. Consider anything that is not fixed or does not remain the same in the animal's environment, but can be placed and removed on a daily basis. For an accurate evaluation of welfare it is essential to distinguish it from environmental complexity.	Is there a formal, written EE program in place and implemented to promote species-specific behavioral opportunities and psychological well-being? Does it include nutritional, social, sensory, cognitive, and occupational environmental enrichment?	A: an EE plan/program is implemented according to a formal, written outline that promotes behavioral opportunities and psychological well-being and all steps are followed, including analysis of the animal's response to EE, as well as the various types of EE. B: an EE plan/schedule is implemented but no observation or analysis of the animal's response to EE is performed, or EEs does not go through an approval process from all areas (veterinary, biology, behavior, nutrition and keepers), or any of the types of EE mentioned in the question are not implemented. C: no EE is performed or it is only performed by the individual will of the keeper or volunteers, without an official plan by the institution.

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TABLE 6 (Continued)

Indicator	Method	Reference	Scoring
Training procedures (MBM)	Interview staff, check documentary records and verify the implementation of a comprehensive and appropriate training plan.	Is there a formal, written animal training plan in place for the animal?	A: training for veterinary and handling maneuvers, cognitive enrichment, strengthening of the human-animal bond and animal exercise is carried out by duly trained personnel, using validated techniques, with a formal, written plan, and in the case of dangerous species, without direct contact between trainer and animal. B: only training for veterinary and handling maneuvers is carried out by duly trained personnel, using validated techniques, without direct contact between trainer and animal in the case of dangerous species, with a formal, written plan. C: training is not carried out and/or is carried out by inadequately trained personnel and/or by means of techniques with doubtful results and/or with direct contact between trainer and animal in the case of dangerous species and/or without a formal and written plan.

RBM, Resources-based measurement; MBM, Management-based measurements.

"Defecation behavior" and "Stool score", together with "Micturition behavior" and "Urine appearance" respectively, due to their physiologic and anatomic features.

Behavioral and mental domain

Ten ABM (Reaction to strangers, Interaction with zookeepers, Exploration, Social, affiliative and maternal-filial behavior, Reproductive behavior, Agonistic behavior, Use of environmental enrichment, Stereotypic behavior, Behavioral diversity, and Space use) were selected to assess the Behavioral and mental domains. Table 8 summarizes the most relevant information provided in the user's manual for assessing behavioral and mental domains, through ten ABM.

Discussion

This study introduced an innovative multi-species animal welfare assessment protocol for wild animals under human care, intended to overcome the use of generic welfare checklists and offer an alternative to challenging and time consuming species-specific tools (24). Ackonc-AWA protocol has several features in common with those of Kagan et al. (16), Brando and Buchanan-Smith [264], Sherwen et al. (3), and Ward et al. (24). They all cover the five domains of animal welfare (31), through indicators that provide information on physical, environmental, behavioral, and social state, as well as husbandry practices, human-animal interactions and individual animal agency. These checklists can be applied to most wild species and, as Ackonc-AWA, fit the prescriptive model (20) since they are helpful in the development of action plans to improve welfare conditions and to set priorities. However, one of the main challenges of working with wildlife is the great diversity of species, with characteristics and needs that are very different from one another (38). Therefore, similar to the work of Asher et al. (27), Clegg et al. (28), Salas et al. (22), Yon et al. (29), and Padalino and Menchetti (30), Ackonc-AWA provides specific indications and descriptions to assist sentinels in the assessment of each indicator. The distinctive feature of Ackonc-AWA is that, notwithstanding its multi-species applicability, it proposes a standardized and detailed guide on the method to adapt, assess and rate each indicator as required by each species.

Therefore, in order to successfully implement the current protocol, prior preparation is a key stage when used on a species for the first time. This includes reviewing the most updated guidelines for the adequate maintenance of the species in captivity and its dietary, health, environmental, behavioral, and affective needs (3, 38). Sometimes this information may not be available, and it becomes necessary to search for information on the species natural history, biology, ecology, diet, sensory systems, natural habitat, social structure, ethogram, activity patterns, and most common health problems and signs of illness,

TABLE 7 Summary of the information provided in the user's manual for assessing health domain.

Indicator (ABM)	Method	Reference	Scoring
Defecation behavior	If the animal is observed during defecation, check body posture, facial expressions and vocalizations.	Does the animal have difficulty or pain during defecation?	A: absence of difficulty or pain during defecation. B: slight difficulty or pain during defecation. C: difficulty or moderate to severe pain during defecation.
Stool score	Observe the characteristics of stool with the aid of the approved fecal condition scales for the species.	Is the stool adequate in terms of consistency, shape, color, frequency of excretion and macroscopic composition (blood, mucus, undigested food, foreign matter)?	A: normal stool, without alterations in any of the aspects to be considered. B: stool with some of the aspects to be considered slightly or incipiently altered. C: stool with some of the aspects to be considered severely altered.
Micturition behavior	If the animal is observed during urination, check body posture, facial expressions and vocalizations.	Does the animal have difficulty or pain to urinate?	A: absence of difficulty or pain on urination. B: slight difficulty or pain during urination. C: difficulty or pain moderate to severe pain during urination.
Urine appearance	Observe the characteristics of urine such as stream fluidity, urine color, frequency and quantity.	Are there any abnormalities in the urine?	A: normal urine, without alterations in any of the aspects to be considered. B: urine with some of the aspects to be considered slightly or incipiently altered. C: urine with two or more of the aspects to be consider altered in a severe way or for several days.
Coat/feathers/ tegument	Observe the characteristics of the skin and the phanerae (features to consider: quantity, brightness and integrity).	Is the plumage/fur/coat/ integument in good condition?	A: good condition of plumage/coat/integument. B: Slight alteration in the quantity or condition of the condition of the coat/plumage/tegument without alteration of its integrity. C: severe alteration in the quantity or condition of the coat/plumage/tegument.
Lesions/injuries	Note the presence of wounds (Pay attention to hair removal, abrasion, redness,swelling, bleeding, abscesses, bruises, presence of flies).	Does the animal appear free of lesions or wounds?	A: absence of lesions and wounds. B: shallow wounds or lesions, small in size and low in number, without infection, suppuration or flies, with mild and short-term effects on animal welfare. C: deep, medium or large wounds or lesions, several in number, with infection, suppuration or flies, with moderate to severe or long-term effects on animal welfare.
Hooves/claws/teeth	According to the species, observe the condition of hooves, claws and teeth as appropriate. Take advantage of situations where the animal is close enough to inspect them (e.g., in training sessions for clinical procedures, when performed).	Is the animal free of overgrowth or lesions on hooves, nails, claws, teeth?	A: hooves/claws/teeth are free of overgrowth and lesions. B: hooves/claws/teeth show mild to moderate overgrowth but are free of lesions. C: hooves/claws/teeth show severe overgrowth and/or lesions.
Locomotion	Observe how the animal moves around the enclosure (features to consider: lameness, reluctance to walk or jump, facial expressions of pain and/or vocalizations while moving)	Does the animal ambulate without difficulty?	A: the animal moves without difficulty or evidence of pain. B: the animal presents mild lameness (grade 1 or 2). C: the animal presents moderate to severe lameness (grade 3 or 4) and/or is reluctance to move and/or experiences evident pain when walking.
Sleep/wakefulness	Observe sleep and activity behaviors at different times of the day.	Does the animal show activity in accordance with the circadian rhythm of its species in nature?	A: the animal's activity is in accordance with the circadian rhythm of the free-living species. C: the animal does not present an activity in accordance with the circadian rhythm of the free-living species.

(Continued)

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TABLE 7 (Continued)

Indicator	Method	Reference	Scoring
Signs of illness	Look for any signs of disease (pay attention to ears, mouth, muzzle, perineal region, respiration, general condition and other anatomical regions or body structures where signs of disease may be evident, depending on the species). Consider signs of disease as identified in the available literature for the species.	Does the animal appear healthy and free of visible signs of disease?	A: the animal appears clinically healthy. B: mild and/or recent symptoms of disease, with minimal effect on animal welfare and/or good prognosis. C: moderate or severe symptoms of disease, or mild but long-standing symptoms, with significant effects on animal welfare and/or unfavorable prognosis.

ABM, Animal-based measurement.

considering the different life stages (25). Some preparation is also needed when assessing an individual for the first time, such as information on enclosure size and design, schematic segmentation of the enclosure according to the biological relevance of each sector, major life history events and medical records. Thus, before applying the Ackonc-AWA protocol for assessing the welfare of an individual, the sentinels should do a crucial (but guided) previous step: to adapt the protocol to the specific welfare-related characteristics and requirements of the target species. By completing a spreadsheet with the optimal conditions for the welfare of the specific species to be evaluated and by adapting the indicators included in the protocol, sentinels would be able to compare them with those observed for the assessed individual and identify potential welfare concerns or needs of improvement.

The need for prior search for information on the species and, if not available, the realization of an ethogram, could take considerable time. This time may be longer or shorter depending on the species, since for some there are husbandry manuals and abundant bibliography, and for others information is very scarce or absent. This prior preparation could be seen as a limitation in comparison to other tools. However, it is important to note that this procedure is done only once at the beginning of the assessment and then the sentinels use the protocol adapted to the species of interest, without the need to go back to the literature for each assessment. In the field trials, the average time used by the sentinels was 51.32 min +/− 29.36 min for the initial form and 58.32 min +/− 23.11 min for the follow-up form. Even with the addition of activity budgets (60 min in total), an increase in the time required for assessment is not expected, as many indicators can be assessed during the same observation. Nevertheless, this should be evaluated in further studies.

Ackonc-AWA implementation cost is low, it is non-invasive/intrusive and takes relatively little time. Although these are all desirable qualities for any animal welfare assessment protocol (39), they could become an essential prerequisite for a welfare evaluation tool intended to be applicable on a daily or weekly basis in institutions with such dissimilar realities, in terms of financial and human resources, as those found in Latin American zoos.

It should be noted that the Ackonc-AWA protocol includes some indicators that can be assessed by close observation and even palpation (i.e., body condition score). This is so that future users of the protocol are able to collect the information in the most practical way for them, as many ABM can be assessed by training and conditioning or during a scheduled veterinary capture. Given that zoos frequently train animals to cooperate in veterinary maneuvers without the need for physical or chemical restraint, and that there is abundant scientific evidence indicating that operant conditioning training is another strategy to improve animal welfare in zoos and the human-animal bond, and is even a form of environmental enrichment (40–42), close observation and hands-on assessment are not discarded.

TABLE 8 Summary of the most relevant information that is provided in the user's manual for assessing behavior and mental domain indicators.

Indicator (ABM)	Method	Reference	Scoring
Reaction to strangers	It should be assessed at any time when the public or zoo staff are unfamiliar to the animal. Assess whether the presence of strangers modifies the occurrence or development of species-specific behaviors, or if signs of fear (e.g., hiding), agonism (e.g., stalking), or habituation (e.g., begging for food or actively seeking interaction) are observed.	Is the animal indifferent to the presence of the public, unfamiliar staff, or observers (if they are not people with whom it has daily contact)?	A: indifferent or positive. C: fear, hidding, aggressiveness, freezing.
Interaction with zookeepers	It should be evaluated any time the animal is in the presence of its keepers. Assess whether this presence modifies the occurrence or development of species-specific behaviors, or if signs of fear (e.g., hiding), agonism (e.g., stalking) or social behaviors (e.g., asking for petting or actively seeking interaction) are observed.	Does the animal have a positive relationship with their keepers?	A: alert, responds to call and commands. B: indifference. C: fear, agonistic behavior.
Exploration	Observe the animal's active exploration of its environment (consider that in addition to wandering, the individual listens, sniffs, licks, or manifests any other component of species-typical exploratory behavior).	Does the animal roam the enclosure and its sorroundings directing their senses to relevant stimuli?	A: exploration is observed. B: exploration is only observed in response to novel stimuli (e.g., environmental enrichment). C: no exploration is observed.
Social, affiliative and maternal-filial behavior	Observe affiliative bonds, such as nurturing and maternal-filial relationship, grooming sessions, or any other component of species-typical social behavior). If the animal is housed in solitary, observe if there are interactions with animals from adjacent enclosures.	Does the individual interact with others in a positive way?	A: positive interaction with other animals. B: indifference or isolation. C: aggressiveness, fear.
Reproductive behavior	Observe the occurrence of reproductive behavior according to the time of year (and species characteristics), proximity of individuals of the same species and different sex, presence of young, courtship behaviors (depending on species: sniffing, urination, marking spray, vocalizations, sensory orientation, etc.). Consider these factors in the different possible contexts (e.g., animals housed in the same enclosure, animals housed in adjacent enclosures with different possibilities of direct contact, and animals housed in nearby enclosures but without direct contact).	Does the animal deploy species-specific reproductive behavior?	A: appetitive and consummatory phases of reproductive behavior are observed in animals housed in the same enclosure during the breeding season. B: incomplete repertoire of reproductive behavior (e.g., substitution behaviors or blank firing) are observed in the breeding season. C: absence of reproductive behavior during the breeding season.
Agonistic behavior	Observe for agonistic interactions and weigh the results. If the animal is housed alone, observe for interactions with animals in adjacent enclosures. Specify in "observations" which individuals are involved and the observed behavior.	Do animals interact with others of the same or related species in a negative way?	A: no more than 3 agonistic interactions marked on the time budget sheets during the 60 min and NO obvious negative effects on animal welfare (e.g., moderate to severe injury or wounding). C: 4 or more agonistic interactions marked on the time budget sheets during the 60 min or <4 WITH obvious negative effects on animal welfare.

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TABLE 8 (Continued)

Indicator	Method	Reference	Scoring
Use of environmental enrichment	Evaluate the animal's response to environmental enrichment (EE) by direct evidence (DE) (visualization of the animal interacting with EE, observing it at the time it is offered) or by indirect evidence (IE) (visualization of the EE or its remains after the animal interacted—or not—with it). Clarify in "observations" which type of EE was observed during assessment.	Is there evidence of interaction with EE?	A: 5 (DE) or 3 (IE), B: 2, 3 or 4 (DE) or 2 (IE), C: 1 (DE or IE).
Stereotypic behavior	Observe for the presence of repetitive, unvarying behaviors with no obvious functional goals. In case B or C, describe in "observations" the behavior in question as detailed as possible.	Does the animal show any abnormal repetitive behavior?	A: the animal does not deploy repetitive behavior. B: repetitive behavior occurs but the pattern retains some variability (it does not always move the same body parts in the same way, it can do it with some variants) and low repeatability (no more than 5 repetitions in a row without stopping). C: the behavior has no variability (always moves the same body parts in the same way) or high repeatability (more than 5 repetitions in a row without stopping).
Behavioral diversity	Complete the "time and space budget sheets" provided by the analyst, in different time slots (morning, noon, afternoon and evening) as specified in the user's manual. Attention! The observer should not assign a score for this indicator. The analyst will be the one to assign the score in consideration of the richness of the behavior (number of behaviors) as well as the uniformity (frequency of each behavior) following the Activity budget method (37).	Does the animal perform species-specific behaviors at natural frequencies and appropriate diversity?	A: Time budget reflects 100% coverage of the functional categories, with no deviations in their proportion as expected for the species. B: the time budget reflects a coverage of between 70 and 100% of the functional categories, with slight deviations in their proportion according to what is expected for the species. C: Time budget reflects a coverage of <70% of the functional categories, with marked deviations in their proportion according to what is expected for the species.
Space use	Complete the "time and space budget sheets" provided by the analyst, in different time slots (morning, noon, afternoon and evening) as specified in the user's manual. Attention! The observer should not assign a score for this indicator. The analyst will be the one to assign the score.	Does the animal make full use of the available space?	A: uses between 85 and 100% of the areas to which it has access. B: uses between 50 and 84% of the sectors of the enclosure to which it has access. C: uses between 0 and 49% of the sectors of the enclosure to which it has access.

ABM, Animal-based measurement.

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However, the protocol has been specifically designed so that contact with the animal is not essential to perform the welfare assessment, and was tested hands-off. This flexibility reinforces the practicality and non-invasiveness attributes of the protocol.

In order to further increase its practicality, Ackonc-AWA was designed in two forms: initial and follow-up. Although, as discussed above, preparation requires some time, once the protocol has been adjusted to the species under study, the follow-up form can be applied as often as necessary, even on a daily basis. Its practicality and low cost of implementation is partly based on the fact that, subject to prior training, it can be applied by the institution's own personnel and done in the context of their daily duties.

In this regard, a core component of developing and using animal welfare assessment tools in zoos is to leverage the experience and expertise of the staff (13, 43, 44). Zoos often have keepers with years of experience working with a particular species, as well as the opportunity to observe individuals over long periods of time and in a variety of contexts. As such, they usually develop skills and abilities to detect and integrate subtle changes in behavior, posture, attitude, expression, or movement (13). In addition, many of the indicators to be assessed are part of their daily tasks, so zookeepers do not need to coordinate with another member of zoo staff the proper moment to do it (e.g., to assess response to environmental enrichment). Furthermore, the inter-observer agreement of ratings performed by zookeepers on zoo animals has been examined and high levels of agreement have been reported (45–48). Therefore, the Ackonc-AWA protocol was conceived to benefit from a systematic collection of information by experienced zookeepers.

Simplicity of implementation is also a key factor for the feasibility of animal welfare assessment protocols. Although the overall feasibility results were positive, adequate training and coaching could be implemented to reduce some of the difficulties encountered by sentinels when filling out the forms in animal welfare assessments. As demonstrated by Rodríguez Ruiz and Heredia Rico (49), training increases reliability of the results and reduces the protocol application time, which becomes relevant since the accuracy of the measurement decreases as the observer gets tired (50). In this study, although inexperienced sentinels received a short training (4 h), the average difficulty values for both forms were relatively low, suggesting that they could be further improved with longer training. This could be explored in future studies.

The indicators most frequently reported as difficult to assess were "Behavioral diversity", "Defecation behavior" and "Micturition behavior". In order to simplify the assessment of "Behavioral diversity", the use of activity budget sheets through focal (individuals) and scan (group) sampling was incorporated, as it is an objective, quantitative and validated method for animal welfare assessment in zoos (37, 51, 52), as well as for "Space use" (53, 54). Regarding "Defecation behavior" and "Micturition behavior", the difficulty could reflect their relatively low frequency of occurrence during brief observation periods. However, we consider that they are indicators of great value for the welfare assessment of animals and their inclusion was deemed necessary. Abnormalities in these two behaviors could be related to somatic conditions and pain or distress, arousal and fear (55). In addition, Ackonc-AWA was intended to be applied by zookeepers, who routinely have the opportunity and the skills to detect these subtle changes in the behavior of the animals in their care (13), which would overcome this constraint. Although the addition of the activity budget sheets could potentially increase the total time required for the assessment, it provides greater robustness in assessing the aforementioned indicators as well as greater flexibility to use the protocol on crepuscular and nocturnal species, through direct or recorded observations. Moreover, the proposed behavioral budget form was designed to reduce time consumption and to be applied in institutions with time constraints, since its interpretation is left to a trained person (analyst) other than the sentinels.

The need for an analyst can also be discussed as a possible disadvantage. Nevertheless, the analysis and interpretation of the information obtained from behavioral budgets has been widely used in zoos, and many of the institutions in Latin America have highly trained personnel within their staff to perform this task.

To assess affective states, Ackonc-AWA proposes a joint approach of the behavioral domain with the mental domain. This is because some affective states are directly or indirectly assessed in this protocol using behavioral indicators. Due to the type of institutions for which this protocol was designed, the importance of assessing affective states in relation to the human-animal bond is emphasized. The effects of the visitors and zookeepers over the animals' experiences and their consequent welfare state are addressed in the protocol through two ABM indicators: Reaction to strangers and Interaction with zookeepers. As stated by Mellor et al. (31), Domain 4 (Behavioral Interactions) is intended to capture behavioral outputs as indices of animals' perceptions of their external circumstances. Hence, the inclusion of Reaction to strangers aimed at evaluating the affective experiences that animals may have when they direct their attention toward unfamiliar people. This could be recognized as behaviors associated with negative states (i.e., freezing, hypervigilance, fear, hiding, and aggressiveness). Behaviors associated with positive states could also be found, as animals actively seek interaction with such strangers. Regarding the indicator Interaction with zookeepers, it is relevant to assess how the animals respond to the staff with whom they are familiar: whether they respond to calls, remain indifferent or display behaviors associated with negative affective states such as those mentioned above.

All of these responses tend to offer an approach to affective states in relation with the interactions that animals and humans have. In the future, further interventions on negative or positive human attributes and attitudes toward animals could be useful to

address this issue from another perspective, in order to acquire a MBM that could operate as a welfare predictor.

The qualitative nature of this protocol may be considered controversial. Observer ratings are scores given to a variable using units of measurement defined by the researchers. Since they involve subjective judgments, some researchers question whether they can be trusted to reflect reality in an unbiased manner (56). However, several studies have shown that observer ratings can be reliable and valid [e.g., (46, 57–60)]. They have been widely employed to assess physical traits [e.g., (61, 62)], health-related variables [e.g., (63–65)], animal personality [e.g., (46, 59)], behavioral patterns [e.g., (45)], and a number of variables relevant to animal welfare [e.g., (66)]. In addition to their practicality, non-invasive nature and low cost (56), observer ratings can be used to integrate multimodal information across time and situations, and for constructs that would otherwise be very difficult to assess [e.g., pain: (65, 67)]. Furthermore, this method seems to be useful for most species that have been tested so far (56). Biases are indeed a risk, especially when the ratings could reflect the observer's or institution's own care of the animals (68–70). Nevertheless, this risk can be minimized by careful wording of the questions to be answered, development of appropriate scales, selection and training of observers, and field testing (56).

With regards to the final assessment results, Ackonc-AWA provides a representation of an animal's welfare and a temporal component that is easy to read and allows tracking changes over time, making it possible to differentiate between problems that affect animal welfare at the current time and those that pose a risk to animal welfare in the medium and long term. As a protocol with a prescriptive approach, it does not give a final numerical result, but looks at each indicator in order to identify potential welfare concerns, which prevents the institution from settling for an acceptable overall result that could be deceiving and could pose a severe threat to animal welfare. For example, a zoo that scores 8 out of 10 might be satisfied with the idea that it has a good overall score and not work on establishing a plan to improve those indicators that were found to be compromised. The situation of these compromised indicators could become chronic and begin to impact negatively on others that were adequate. On the other hand, by letter-marking indicators it is easy to identify those that require immediate resolution and establish a prioritization plan. The proposed 3-point scale score would facilitate a fast and practical prioritization of the identified welfare concerns, and to tag the more urgent correction actions.

The implementation of Ackonc-AWA in zoos could be very useful for decision-making within the ethical frameworks of compassionate conservation and conservation welfare, by evaluating the impact of different actions and situations, and guiding future decisions, so to ensure that *ex situ* conservation efforts do not harm (or do as little as possible) the welfare of individuals (8, 71).

Conclusion

This study aimed to develop, test in the field, and describe an animal welfare assessment protocol for wild animals under human care, that can be applied on a daily basis, noninvasively, and at a low cost, under the prescriptive model. Therefore, a protocol structured in two forms (one exhaustive and other for routine use) was tested on 14 species of different taxa housed in a zoo in Argentina. Representatives from different areas of the institution as well as 3 of the authors participated in the test. It was possible to demonstrate the feasibility and test-retest reliability of the protocol. However, due to time limitations of the institution staff, its inter-observer reliability has yet to be tested.

As a result of this process, Ackonc-AWA, a multidimensional protocol for welfare assessment in multiple animal species under human care, was obtained. This proposal offers an intermediate solution between protocols that are easy to apply yet rely entirely on the judgment of the assessors, and validated but species-specific protocols that are useful only for assessing the species for which they were developed.

Further applications of the described welfare assessment tool in other species and different institutional contexts will reinforce the validation of the proposed measurements and allow the systematic and routine evaluation of animal welfare in zoos.

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.

Ethics statement

Ethical review of the project was requested to the Institutional Committee for the Care and Use of Laboratory Animals (CICUAL) of the Faculty of Veterinary Sciences of the University of Buenos Aires, and a review exemption was granted given the observational nature of the project. The study focused on the non-invasive/intrusive assessment of animal welfare, so no interventions of any kind were carried out on the animals. There were no potential adverse effects, nor foreseeable risks or hazards associated with this project, with regards to animal, plant and/or human wellbeing. The participation of zoo staff in this study was completely voluntary and written informed consent was obtained from all participants. The survey responses were strictly confidential and data from this research was reported only in the aggregate. The information was coded and remains confidential.

Author contributions

DR, AF, and LR contributed to the elaboration of the first drafts of the protocol, interviewed the zoo staff, met with the zoo staff/focus group to test validity, designed the on-field test, trained the staff as sentinels, organized the scheduled observations for all the sentinels, did field work as sentinels, analyzed the results of the observations including feasibility results, and elaborated the Ackonc-AWA protocol. DR contacted and coordinated actions with the institution where the pilot test was conducted and wrote and edited the first draft of this manuscript. LR and AF wrote sections of the manuscript and edited the first draft and manuscript. CB was responsible for the statistical analysis of the results for test-retest reliability and edited the manuscript. OT-P collaborated as the main counselor for methodological aspects of the study and edited the manuscript. All authors contributed to manuscript revision, and read and approved the submitted version.

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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.2022.1033821/full#supplementary-material

References

1. Fraser D. Toward a synthesis of conservation and animal welfare science. *Animal Welfare.* (2010) 19:121–4. Available online at: https://www.wellbeingintlstudiesrepository.org/conbawel/1/

2. Swaisgood RR. The conservation-welfare nexus in reintroduction programmes: a role for sensory ecology. *Animal Welfare.* (2010) 19:125–37.

3. Sherwen SL, Hemsworth LM, Beausoleil NJ, Embury A, Mellor DJ. An animal welfare risk assessment process for zoos. *Animals.* (2018) 8:130–146. doi: 10.3390/ani8080130

4. Skovlund CR, Kirchner MK, Moos LW, Alsted N, Manteca X, Tallo-Parra O et al. A critical review of animal-based welfare indicators for polar bears (*Ursus maritimus*) in zoos: identification and evidence of validity. *Animal Welfare.* (2021) 30:1–18. doi: 10.7120/09627286.30.1.001

5. Ramp D, Bekoff M. Compassion as a practical and evolved ethic for conservation. *Bioscience.* (2015) 65:323–7. doi: 10.1093/biosci/biu223

6. Branco ARV, Soriano VS, Schnaider MA, Molento CFM. Compassionate conservation: concept and applications. *Arch Vet Sci.* (2017) 22:116–30. doi: 10.5380/avs.v22i4.56938

7. Papastavrou V, Leaper R, Lavigne D. Why management decisions involving marine mammals should include animal welfare. *Mar Pol.* (2017) 79:19–24. doi: 10.1016/j.marpol.2017.02.001

8. Beausoleil NJ, I am a compassionate conservation welfare scientist: Considering the theoretical and practical differences between Compassionate Conservation and Conservation Welfare. *Animals.* (2020) 10:257. doi: 10.3390/ani10020257

9. Bacon H, Vigors B, Shaw DJ, Waran N, Dwyer CM, Bell C. Zookeepers–The most important animal in the zoo? *J Appl Animal Welf Sci.* (2021) 1–13. doi: 10.1080/10888705.2021.2012784

10. Walker M, Diez-Leon M, Mason G. Animal welfare science: Recent publication trends and future research priorities. *Int J Compar Psychol.* (2014) 27:80–100. doi: 10.46867/ijcp.2014.27.01.03

11. Rose PE, Brereton JE, Rowden LJ, de Figueiredo RL, Riley LM. What's new from the zoo? An analysis of ten years of zoo-themed research output. *Palgrave Commun.* (2019) 5:1–10. doi: 10.1057/s41599-019-0345-3

12. Binding S, Farmer H, Krusin L, Cronin K. Status of animal welfare research in zoos and aquariums: Where are we, where to next? *J Zoo Aquar Res.* (2020) 8:166–74. doi: 10.19227/jzar.v813.505

13. Whitham JC, Wielebnowski N. Animal-based welfare monitoring: using keeper ratings as an assessment tool. *Zoo Biol.* (2009) 28:545–60. doi: 10.1002/zoo.20281

14. Registro Publico Unico De Operadores De Fauna Silvestre. *Resolución de 2021 del Ministerio de Ambiente y Desarrollo Sustentable de la Nación Argentina. N° 170/21.* Buenos Aires: Boletín oficial de la República Argentina (2021).

15. Conservación de la Fauna. *Programa de Reconversión de Zoológicos. Resolución de 2017 del Ministerio de Ambiente y Desarrollo Sustentable de*

la Nación Argentina. N° 311/17. Buenos Aires: Boletín oficial de la República Argentina (2017).

16. Kagan R, Carter S, Allard S, A. universal animal welfare framework for zoos. *J Applied Animal Welfare Sci.* (2015) 18:S1–S10. doi: 10.1080/10888705.2015.1075830

17. Mason GJ, Mendl M. Why is there no simple way of measuring animal welfare? *Animal Welfare.* (1993) 2:301–19.

18. Fraser D. Science, values and animal welfare: exploring the inextricable connection. *Animal Welfare.* (1995) 4:103–17.

19. OIE. *OIE—World Organization for Animal Health*. (2018). Available online at: http://www.oie.int/es/bienestar-animal/el-bienestar-animal-de-un-vistazo/ (accessed April 13, 2021).

20. Botreau R, Bonde M, Butterworth A, Perny P, Bracke MBM, Capdeville J, et al. Aggregation of measures to produce an overall assessment of animal welfare. Part 1: a review of existing methods. *Animals.* (2007) 1:1179–87. doi: 10.1017/S1751731107000535

21. Department for Environment, Food & Rural Affairs (DEFRA). *Zoos Expert Committee Handbook. Chapter 4: Animal Welfare and its Assessment in Zoos.* (2012). Available online at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/69611/pb13815-zoos-expert-committee-handbook1.pdf (accessed April 13, 2021).

22. Salas M, Manteca X, Abáigar T, Delclaux M, Enseñat C, Martínez-Nevado E et al. Using Farm Animal Welfare Protocols as a Base to Assess the Welfare of Wild Animals in Captivity—Case Study: Dorcas Gazelles (Gazella dorcas). *Animals.* (2018) 8:111–9. doi: 10.3390/ani8070111

23. Welfare Quality Network. *Welfare Quality R Project.* (2009). Available online at: http://www.welfarequality.net/en-us/news/assessment-protocols/ (accessed June 9, 2022).

24. Ward SJ, Williams E, Groves G, Marsh S, Morgan D. Using zoo welfare assessments to identify common issues in developing country zoos. *Animals.* (2020) 10:2101. doi: 10.3390/ani10112101

25. Brando S, Buchanan-Smith HM. The 24/7 approach to promoting optimal welfare for captive wild animals. *Behav Processes.* (2018) 156:83–95. doi: 10.1016/j.beproc.2017.09.010

26. Carlstead K, Mench JA, Meehan C, Brown JL. An epidemiological approach to welfare research in zoos: The elephant welfare project. *J Applied Animal Welfare Sci.* (2013) 16:319–37. doi: 10.1080/10888705.2013.827915

27. Asher L, Williams E, Yon L. *Developing Behavioural Indicators, as Part of a Wider Set of Indicators, to Assess the Welfare of Elephants in UK Zoos. Research Report for External Body*. Bristol: DEFRA (Department for Environment, Food & Rural Affairs) (2015). 214 p. Report No.: Project WC1081: Final Report.

28. Clegg IL, Borger-Turner JL, Eskelinen HC. C-Well: The development of a welfare assessment index for captive bottlenose dolphins (Tursiops truncatus). *Animal Welfare.* (2015) 24:267–82. doi: 10.7120/09627286.24.3.267

29. Yon L, Williams E, Harvey ND, Asher L. Development of a behavioural welfare assessment tool for routine use with captive elephants. *PLoS ONE.* (2019) 14:e0210783. doi: 10.1371/journal.pone.0210783

30. Padalino B, Menchetti L. The first protocol for assessing welfare of camels. *Front Vet Sci.* (2021) 7:1230. doi: 10.3389/fvets.2020.631876

31. Mellor DJ, Beausoleil NJ, Littlewood KE, McLean AN, McGreevy PD, Jones B, Wilkins C. The 2020 five domains model: including human–animal interactions in assessments of animal welfare. *Animals.* (2020) 10:1870. doi: 10.3390/ani10101870

32. Scott EM, Nolan AM, Fitzpatrick JL. Conceptual and methodological issues related to welfare assessment: a framework for measurement. *Acta Agric Scand A Anim Sci.* (2001) 51:5–10. doi: 10.1080/090647001316922983

33. Battini M, Vieira A, Barbieri S, Ajuda I, Stilwell G, Mattiello S. Invited review: animal-based indicators for on-farm welfare assessment for dairy goats. *J Dairy Sci.* (2014) 97:6625–48. doi: 10.3168/jds.2013-7493

34. Landis JR, Koch GG. An application of hierarchical kappa-type statistics in the assessment of majority agreement among multiple observers. *Biometrics.* (1977) 33:363–74. doi: 10.2307/2529786

35. Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW. *InfoStat versión 2020*. Argentina: Centro de Transferencia InfoStat, FCA, Universidad Nacional de Córdoba. Available online at: http://www.infostat.com.ar (accessed on August 1, 2022).

36. Lowry, R. *VassarStats: Website for Statistical Computation* (1998–2022). Available online at: http://vassarstats.net/ (accessed August 1, 2022).

37. Miller LJ, Vicino GA, Sheftel J, Lauderdale LK. Behavioral diversity as a potential indicator of positive animal welfare. *Animals (Basel).* (2020) 10:1211. doi: 10.3390/ani10071211

38. Hill SP, Broom DM. *Measuring zoo animal welfare: theory and practice. Zoo Biol.* (2009) 28:531–44. doi: 10.1002/zoo.20276

39. Pedrazzani AS, Quintiliano MH, Bolfe F, Sans ECDO, Molento CFM. Tilapia on-farm welfare assessment protocol for semi-intensive production systems. *Front Vet Sci.* (2020) 7:606388. doi: 10.3389/fvets.2020.606388

40. Laule GE. Positive reinforcement training and environmental enrichment: enhancing animal well-being. *J-Am Vet Med Assoc.* (2003) 223:969–72. doi: 10.2460/javma.2003.223.969

41. Melfi V. Is training zoo animals enriching? *Appl Animal Behav Sci.* (2013) 147:299–305. doi: 10.1016/j.applanim.2013.04.011

42. Fernandez EJ, Martin AL. Animal training, environmental enrichment, and animal welfare: a history of behavior analysis in zoos. *J Zool Bot Gardens.* (2021) 2:531–43. doi: 10.3390/jzbg2040038

43. Barrows M. Welfare assessment in zoo animals. *Vet Rec.* (2017) 181:141–2. doi: 10.1136/vr.j3583

44. Riggio G, Pirrone F, Lunghini E, Gazzano A, Mariti C. Zookeepers' perception of zoo canid welfare and its effect on job satisfaction, worldwide. *Animals.* (2020) 10:916. doi: 10.3390/ani10050916

45. Carlstead K, Mellen J, Kleiman DG. Black rhinoceros (Diceros bicornis) in U.S. zoos: I. Individual behavior profiles and their relationship to breeding success. *Zoo Biol.* (1999) 18:17–34. doi: 10.1002/(SICI)1098-2361(1999)18:1<17::AID-ZOO4>3.0.CO;2-K

46. Wielebnowski NC. Behavioral differences as predictors of breeding status in captive cheetahs. *Zoo Biol.* (1999) 18:335–49. doi: 10.1002/(SICI)1098-2361(1999)18:4<335::AID-ZOO8>3.0.CO;2-X

47. Wielebnowski NC, Fletchall N, Carlstead K, Busso JM, Brown JL. Noninvasive assessment of adrenal activity associated with husbandry and behavioral factors in the North American clouded leopard population. *Zoo Biol.* (2002) 21:77–98. doi: 10.1002/zoo.10005

48. Whitham JC, Wielebnowski N. New directions for zoo animal welfare science. *Appl Anim Behav Sci.* (2013) 147:247–60. doi: 10.1016/j.applanim.2013.02.004

49. Rodríguez Ruiz Y, Heredia Rico JJ. Confiabilidad ínter-observador del método de evaluación de riesgo individual. *Hacia la Promoción de la Salud.* (2013) 18:41–56.

50. Méndez Ravanal MC. *Aplicabilidad de un protocolo de medición de bienestar animal creado por el proyecto Welfare Quality® en gallinas de postura comercial en jaulas.* (2010). Available online at: https://repositorio.uchile.cl/handle/2250/131311

51. Antonenko TV, Medvedeva JE, Panchuk KA. The influence of olfactory stimulation on the welfare of big cats in captivity. *Ukr J Ecol.* (2017) 7:134–8. doi: 10.15421/2017_96

52. Huettner T, Dollhaeupl S, Simon R, Baumgartner K, von Fersen L. Activity budget comparisons using long-term observations of a group of bottlenose dolphins (Tursiops truncatus) under human care: Implications for animal welfare. *Animals.* (2021) 11:2107. doi: 10.3390/ani11072107

53. Ross SR, Schapiro SJ, Hau J, Lukas KE. Space use as an indicator of enclosure appropriateness: a novel measure of captive animal welfare. *Appl Anim Behav Sci.* (2009) 121:42–50. doi: 10.1016/j.applanim.2009.08.007

54. Wark JD, Cronin KA, Niemann T, Shender MA, Horrigan A, Kao A, et al. Monitoring the behavior and habitat use of animals to enhance welfare using the ZooMonitor app. *Anim Behav Cogn.* (2019) 6:158–67. doi: 10.26451/abc.06.03.01.2019

55. Smulders D, Verbeke G, Mormède P, Geers R. Validation of a behavioral observation tool to assess pig welfare. *Physiol Behav.* (2006) 89:438–47. doi: 10.1016/j.physbeh.2006.07.002

56. Meagher RK. Observer ratings: Validity and value as a tool for animal welfare research. *Appl Anim Behav Sci.* (2009) 119:1–14. doi: 10.1016/j.applanim.2009.02.026

57. Wemelsfelder F. The scientific validity of subjective concepts inmodels of animal welfare. *Appl Anim Behav Sci.* (1997) 53:75–88. doi: 10.1016/S0168-1591(96)01152-5

58. Carlstead K, Shepherdson D, Sheppard C, Mellen J, Bennet C. *Constructing Behavioural Profiles for Zoo Animals: Incorporating Behavioural Information into Captive Population Management*. Silver Spring, MD: American Zoo and Aquarium Association's Behaviour and Husbandry Advisory Group and Oregon Zoo (2000).

59. Gosling SD. From mice to men: what can we learn about personality from animal research? *Psychol Bull.* (2001) 127:45–86. doi: 10.1037/0033-2909.127.1.45

60. Diesel G, Brodbelt D, Pfeiffer DU. Reliability of assessment of dogs' behavioural responses by staff working at a welfare charity in the UK. *Appl Anim Behav Sci.* (2008) 115:171–81. doi: 10.1016/j.applanim.2008.05.005

61. Jefferies BC. Body condition scoring and its use in management. *Tasmanian J Agr*. (1961) 32:19–21.

62. Russel AJF, Doney JM, Gunn RG. Subjective assessment of body fat in live sheep. *J Agric Sci*. (1969) 72:451–4. doi: 10.1017/S0021859600024874

63. Kestin SC, Knowles TG, Tinch AE, Gregory NG. Prevalence of leg weakness in broiler chickens and its relationship with genotype. *Vet Rec*. (1992) 131:190–4. doi: 10.1136/vr.131.9.190

64. Flower FC, Weary DM. Effect of hoof pathologies on subjective assessments of dairy cow gait. *J Dairy Sci*. (2006) 89:139–46. doi: 10.3168/jds.S0022-0302(06)72077-X

65. Hawkins P. Recognizing and assessing pain, suffering and distress in laboratory animals: a survey of current practice in the UK with recommendations. *Lab Anim*. (2002) 36:378–95. doi: 10.1258/0023677023203 89044

66. Wemelsfelder F. How animals communicate quality of life: the qualitative assessment of behaviour. *Anim Welf*. (2007) 16:25–31.

67. Rutherford KMC. Assessing pain in animals. *Anim Welf*. (2002) 11:31–53.

68. Matsumoto D, Juang L. *Culture and Psychology*. Toronto, CA Thomson Learning—Wadsworth (2004).

69. Furr RM, Bacharach VR. *Psychometrics: An Introduction*. Thousand Oaks: CA: Sage Publications, Inc. (2008), 246–250.

70. Wyse CA, McNie KA, Tannahil VJ, Murray JK, Love S. Prevalence of obesity in riding horses in Scotland. *Vet Rec*. (2008) 162:590–1. doi: 10.1136/vr.162.18.590

71. Learmonth, MJ. Human–animal interactions in zoos: What can compassionate Conservation, conservation welfare and duty of care tell us about the ethics of interacting, and avoiding unintended consequences?. *Animals*. (2020) 10:2037. doi: 10.3390/ani10112037

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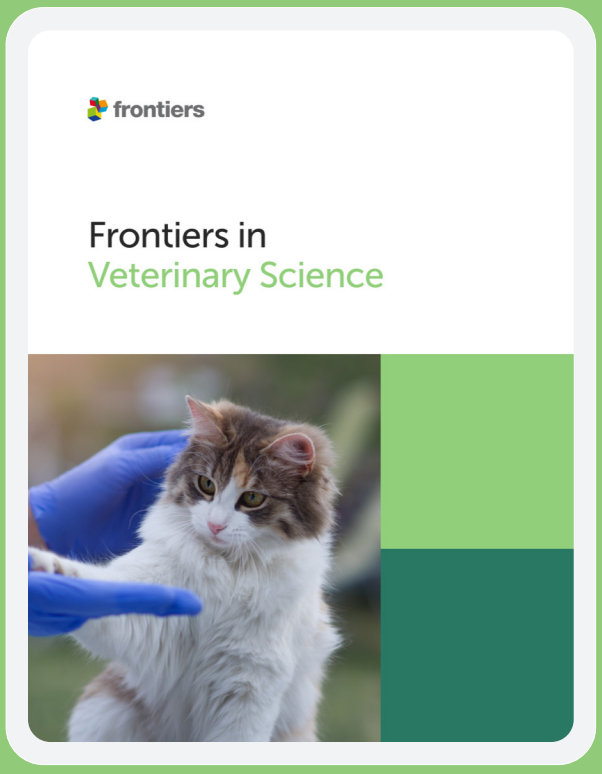

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