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The interpretation of emotions in small ruminants

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The interpretation of emotions in small ruminants

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Mattiello, S., **Celozzi, S.**, Soli, F.M., & Battini, M. (2024) Exploring positive welfare measures: preliminary findings from a prototype protocol in loose housing dairy cattle farms. *Frontiers in Veterinary Science*, 11:1368363. Doi: 10.3389/fvets.2024.1368363

General abstract

Ensuring that animals have a life worth living means not only reducing their exposure to stressful and/or negative situations, but above all ensuring that they can experience predominantly positive affective states and emotions. The aim of this thesis was to improve human knowledge of how small ruminants (goats, *Capra hircus*, and sheep, *Ovis aries*) express emotions, with a particular focus on the acoustic features of vocalisations that can be used as animal-based indicators of the affective states of these animals. To pursue this goal, a multidisciplinary approach was adopted in this thesis, ranging from the study of the direct perception and interpretation of vocalisations by humans to the use of traditional acoustic analysis to decipher the emotional content of vocalisations, through the extraction of acoustic features and the application of statistical models, and their practical application for the development of up-to-date Precision Livestock Farming (PLF) technologies.

The general introduction of the thesis provided an overview of the state of the art of research on small ruminant emotions and included a review paper on the topic of human-goat relationship. In fact, in farmed animals like sheep and goats, the quality of the human-animal relationship (HAR) depends on the emotional connotations of the interactions that occur between the two parties. The review focused on how humans and goats communicate, which factors influence this relationship, which methods are available to measure it, and how it can be improved. The review showed that certain interspecies communication channels, particularly the acoustic ones, have received little attention so far, and highlighted the importance of early, frequent and positive interactions with goats to improve HAR, and the role of some socio-demographic variables, such as gender, in influencing behaviour and attitude towards these animals. The review also highlighted the poor feasibility of the validated measures available for farmers to use for self-assessment of HAR, and suggested that farmers' sense of commitment to goats could be improved by increasing their awareness of goats' ability to express emotional states.

The first research chapter presented the development of a wireless acoustic sensor network for the continuous and non-invasive monitoring of goat vocalisations. The design, architecture and implementation of the system were presented, together with the hierarchical classification of goat vocalisations adopted for the manual annotation of these acoustic signals. In this context, the study also presented the development of a web-based annotation tool of goat vocalisations, to allow the association of labels, namely the context of emissions, to goat vocalisations. Finally, the study presented the development of a smartphone application to alert farmers and inform them of the emotional state of their goats.

The second research chapter is an investigation of human perception of goat emotions based on their vocalisations. This human ability was investigated taking into account the respondents' previous experience with goats, their level of empathy towards these animals, and other individual characteristics that may influence the recognition of animal emotions, and by considering the dimensional framework of animal emotions, which considers them as characterised by the dimensions of valence (positive *vs* negative) and arousal (low *vs* high). It was found that humans are able to recognise the emotional dimension of valence in goat vocalisations, as suggested by the consistency of the qualitative description of these vocalisations with their context of emission. The study also revealed a bias in the human ability to classify goat vocalisations emitted in negative contexts, presumably due to the fact that signals emitted in negative emergency situations carry more important and urgent information than those emitted in positive situations. The human ability to classify goat vocalisations was found to be influenced by the level of experience with these animals, with people with frequent direct contact with goats showing higher correct classification rates of the context of emission. Other individual characteristics, such as the level of empathy towards goats, seemed to improve the association of bleats to the correct emission contexts, although further studies are needed to clarify the role of empathy, attitudes and their underlying factors. Having children, gender and university education were not found to influence the human ability to interpret goat vocalisations.

In the third research chapter, acoustic, behavioural and physiological markers of negative arousal in lambs were investigated in a two-phases isolation test (partial *vs* full) designed to induce negative emotions, but characterised by different intensities of emotional arousal. Results showed that lambs produced more vocalizations per time unit and with higher frequencies (energy quartiles, formants, fundamental frequency) and Wiener entropy when experiencing negative arousal. These acoustic features were also found to increase with the bodily activation of the individual (namely the level of activity) as the arousal increased. Although the use of thermography as a method of detecting emotional states in animals seems promising, the results using temperature as a physiological indicator were inconsistent. Finally, the individual level of sociability did not affect the behavioural and physiological response of lambs to isolation, while an effect of the size of the lamb was found; both factors deserve further investigation.

Overall, the results of these studies advance the understanding of the expression of emotions in small ruminants, with practical implications for improving the quality of the human-animal relationship and the level of animal welfare, and highlight an important and potential role for PLF technologies in the automated collection and interpretation of emotions in sheep and goats.

Introduction

1. Emotions

1.1 Defining the concept of emotions in animals

The ‘Expression of Emotions in Man and Animals’, written by Charles Darwin in 1872, not only represents the first study of emotions in humans, but also suggests that they are not unique to this species, being also present in non-human animals (Ekman, 2009). However, the study of animals’ feelings and emotions is difficult, especially because of the impossibility to get access to animal consciousness. What we know about animal emotions mostly derives from a process of inference from human emotion concepts, theories and indicators (Mendl et al, 2022).

Nevertheless, the scientific community has shown a growing interest in animal emotions, especially in the last fourteen years (Figure 1), which has led to the need for the use of a clear and common terminology, starting with the definition of the concept of ‘emotion’ (LeDoux, 2016).

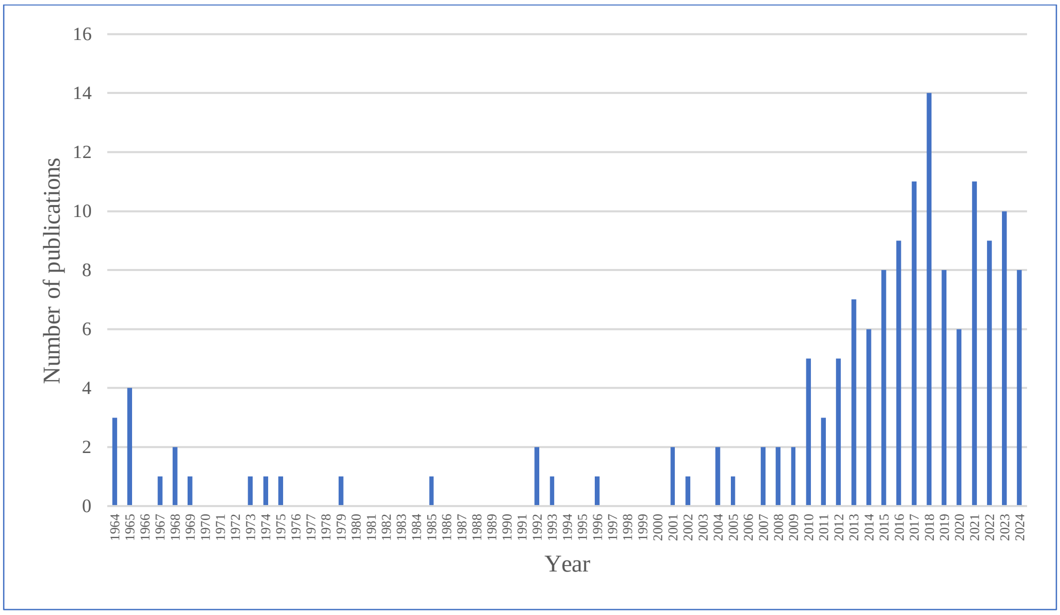

Figure 1. Number of publications from 1964 to date by searching the key words ‘emotion’, or ‘affective’ or ‘feeling*’ in the articles’ title. Articles searched in Web of Science (29/07/2024) in the following journals: Applied Animal Behaviour Science, Animal Welfare, Journal of Applied Animal Welfare Science, Animal Behaviour, Ethology, Behaviour, Animal Cognition*

According to LeDoux (2016), emotion is a neural (cerebral) state, a mental process distinct but inseparable from cognition, and Panksepp (1998) defines it as an affective state of short duration, resulting from rewarding or punishing stimuli or events (which can increase or decrease the individual’s quality of life, respectively). In the human species, emotions are consciously experienced by the individual (“subjective feelings”) and can be referred to through language (linguistic self-report), using labels such as "happy", "sad", "depressed". These conscious experiences are characterised by a valence, or degree of pleasantness: good *vs* bad; pleasant *vs* unpleasant; rewarding *vs* punishing (Izard, 2009).

From an anatomical point of view, Panksepp (2011) described how "the emotional feelings of the primary process originate from ancient caudal and medial subcortical regions of the brain". More recently, emotions have been associated with the presence of other neural structures, such as the cerebral cortex (LeDoux and Brown, 2017) and the midbrain (Barron and Klein, 2016).

1.2 The evolutionary basis of the development of emotions

From an evolutionary perspective, emotions have evolved to enable individuals to manage their decision and behavioural responses for the purpose of increased survival by obtaining resources and avoiding danger (Izard, 2009). Recently, a model that integrates animal affect and decision making has been proposed, in which short-term ("emotion-like") states enable short-term decision making through processes such as affective options, affective predictions, and affective outcomes. Long-term (mood) states, on the other hand, drives decisional behavioural choices (Mendl and Paul, 2020). The adaptive function of emotions enables animals to respond to environmental challenges. In mammals, this function is performed by basic discrete emotional systems, such as fear, anger, and panic, which are located in the neural systems of specific brain areas and represent the fundamental building blocks of all emotional reaction (Panksepp, 2011). In social species, emotions are conveyed through different communication channels. The ability to perceive the emotions expressed by another subject makes it possible to predict its behaviour and potentially be influenced by its emotional state (emotional contagion; Briefer., 2018). In this way, emotions act as a regulatory element in social interactions (Panksepp, 2009).

1.3 Do animals consciously perceive emotions?

Whether or not animals are capable of perceiving emotions, i.e., subjectively experiencing them, is the subject of much debate in the scientific community, and there is currently no definite answer to this question (Mendl et al., 2010). Mendl and Paul (2020) use the suffix 'like' when referring to the emotions and moods of animals, precisely to indicate the inability of humans to verify the conscious component of animal emotional states. Several authors do not deny the possibility that animals can consciously experience emotions, but argue that the current scientific evidence does not allow this to be proven (LeDoux, 2016). Dawkins (2015) argues that this inability would be at the root of the paradox of animal welfare science, which, to be defined as such, should be able to study animal consciousness, something biologists currently find difficult, if not impossible to do.

Among the proponents of the conscious perception of emotions in non-human animals is Wemelsfelder, who argues that it is possible to observe directly how animals experience a situation ("subjective experience or discrete emotions") through the way they express their behaviour ("expressive actions"; Wemelsfelder, 1997). As mentioned above, humans use language to express emotions, and this ability would be at the basis of their conscious perception. However, Ekman (2009) argues that words cannot be considered a *conditio sine qua non* of emotions, as this would be like saying, for example, that infants are emotionless before they acquire language.

The ability to experience "primal affective feelings" would be common to all vertebrates. In mammals, there are also affective similarities that attest to the presence of "conserved primary-process emotional mechanisms of the brain that can engender reward and punishment functions in learning tasks" (Panksepp, 2011). On 7 July 2012, an international group of cognitive neuroscientists, neuropharmacologists, neurophysiologists, neuroanatomists and computational neuroscientists met at the University of Cambridge to discuss the neuroanatomical, neurochemical and neurophysiological substrates underlying consciousness and associated behaviour in humans and animals. The "Cambridge Declaration on Consciousness" was written, proclaimed and signed by the participants on the day of the conference. The declaration states that all vertebrates, including fish and reptiles, possess the neurological substrates of consciousness. Reference is also made to invertebrates (decapod crustaceans, cephalopod molluscs and insects), although only octopuses are specifically mentioned in the declaration, as there was a scientific presentation on them at the conference (Low, 2012).

In March 2023, I had the opportunity to attend the PhD student course "Fundamentals of Animal Emotion", organised by Wageningen University & Research. In the lecture by Professor Mike Mendl of the University of Bristol, that introduced the concept of emotion, much space was given to the question of the capacity for conscious experience in animals, which would depend on the presence of the following structures, functions and capacities:

Presence of relevant neural structures from which conscious experiences arise

- Cortex (LeDoux and Brown, 2017)
- Subcortex (Panksepp, 2005)
- Midbrain (Merker, 2008; Barron and Klein, 2016)

Presence of neural function correlated with human consciousness

- Thalamocortical signalling (Edelman and Seth, 2009)
- Widespread and dispersed cortical activity (Edelman and Seth, 2009)
- 'Ignition' of neuronal communication between many brain areas (parietal and prefrontal circuits; Dehaene, 2014)

Possession of cognitive abilities that may imply conscious processing

- Episodic memory and future planning (self-representation over time; (Edelman & Seth 2009)
- Metacognition or higher-order consciousness (being aware of what one knows; (Edelman & Seth 2009)
- Self-concept and theory of mind (Edelman & Seth 2009).

This suggests that not only a neurological but also a cognitive component exists which underlies the conscious perception of emotions (Birch et al., 2020). As for the possibility of animals to be able to consciously plan ahead, a study by Pfeiffer and Foster (2013) showed that the activation trajectories of hippocampal place cells of stationary rats were able to predict the path the subject would take, suggesting the possibility of conscious planning.

Birch et al. (2020) proposed a multidimensional framework in which variation in interspecies states of consciousness are explained by five dimensions (Figure 2):

1) **Perceptual richness** – the level of detail of conscious perception, specific to each sense modality
2) **Evaluative richness** – the ability to assess the valence of stimuli for decision-making and motivational trade-offs
3) **Integration of perspective at a time** – It refers to the unification or the disunification of conscious experience
4) **Integration across time** – It refers to the possibility of conscious experience flowing through time, which is taken for granted in the human species (mental time travel and planning for the future)
5) **Self-consciousness** – It refers to perceiving oneself as distinct from the world

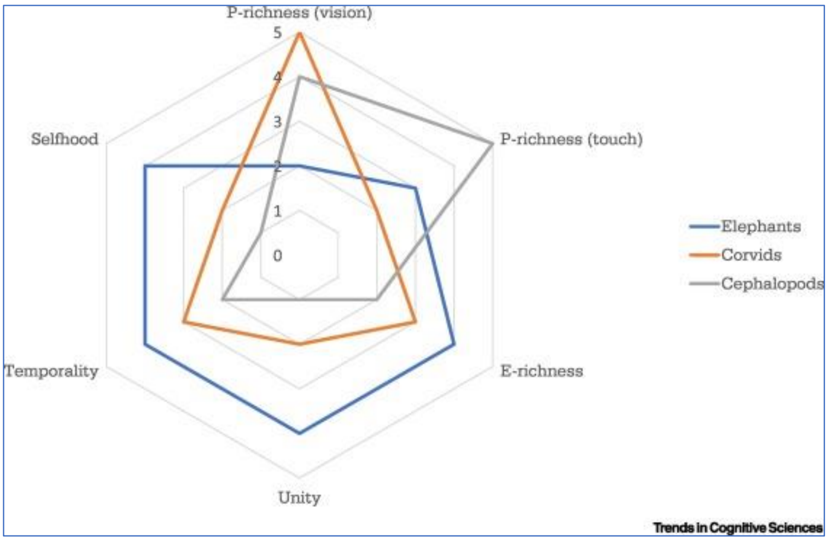

Figure 2. Multidimensional framework of the dimensions of animal consciousness (from Birch et al., 2020)

This framework makes it possible to identify a profile of consciousness specific to each species, but it does not allow to say whether one species is more or less conscious than another.

Since the Cambridge Declaration of Consciousness, further scientific contributions have been made about sentience in invertebrates such as insects, cephalopods and decapods (Barron and Klein, 2016; Birch et al., 2021). Recently, Birch et al (2021) developed a framework for evaluating scientific evidence on sentience in cephalopod molluscs and decapod crustaceans based on eight criteria:

1) Possession of nociceptors
2) Possession of integrative brain regions
3) Connections between nociceptors and integrative brain regions
4) Responses affected by potential local anaesthetics or analgesics
5) Motivational trade-offs that show a balancing of threat against opportunity for reward
6) Flexible self-protective behaviours in response to injury and threat
7) Associative learning that goes beyond habituation and sensitisation
8) Behaviour that shows the animal values local anaesthetics or analgesics when injured.

All these criteria must be considered in order to state whether an animal species belonging to the cephalopods or decapods can be defined as sentient. Each criterion has a confidence level (very high, high, medium, low, very low or no confidence) based on the number of scientific papers, their reliability and quality, and describes the confidence in asserting that an animal species meets or does not meet the criterion. If a species meets seven or more criteria with a high or very high level of confidence, there is strong evidence of sentience for that species. If a species meets five or more criteria with a high or very high level of confidence, there is strong evidence for sentience, and the same level of confidence in meeting three or more criteria leads to an overall judgement of substantial evidence for sentience (Birch et al., 2021).

1.4 Affective states, emotions and mood: are they the same thing?

As mentioned in Chapter 1.1, the use of clear and common terminology seemed to be a necessity for animal emotion researchers. We are indebted to Mendl and Paul (2020) for the first (and incredibly recent) attempt to propose a set of definitions in this field (Table 1).

Table 1. Terminology used in the field of animal emotions (from Mendl and Paul, 2020; definition of "Core affect" in humans by Russell, 2003; definition of "Sentience" by Webb, 2019)

Term	**Definition**
"Emotional/affective states"	States that have valence. They include both emotions and moods, but also the valence components of sensations (e.g., the unpleasantness of pain). They are consciously experienced but are characterized also by non-conscious components (e.g., behavioural indicators, neural changes)
"Emotions"	Short-term, object-focused affective states. The use of the suffix "like" (e.g., "emotion-like") refers to our agnosticism about consciousness in non-human animals
"Moods"	Long-term "free-floating" affective states not due to specific objects or events
"Core affect"	Conceptual framework of human emotions that are linguistically reported. "Core affect space" is a bidimensional space defined by two dimensions, i.e. valence and arousal. In animals, core affect is used with the same meaning as for humans, but without implying conscious experience. In humans core affect is defined as "a neurophysiological state that is consciously accessible as a simple, nonreflective feeling that is an integral blend of hedonic (pleasure–displeasure) and arousal (sleepy–activated) values" (Russell, 2003)
"Feelings"	The subjective component of affective states. It refers to the possibility of consciously experiencing these states
"Sentience"	The ability to feel pain and pleasure (Webb, 2019)

It is clear from this list of definitions that valence, or the degree of pleasantness, is the key characteristic of affective states, which includes both emotions and moods. Moods are generated by the accumulation of previous experiences, whether positive (i.e., rewarding events) or negative (i.e., punishing events). They can be seen as a "relatively pure form of core affect", as they are not characterised by the actions and reactions that result from the process of evaluating the contexts underlying discrete emotions (Mendl et al., 2010). Recent studies in the humans suggest that it is not only the accumulation of experiences that is important, but also the trajectory of these experiences, i.e., how they evolve over time, including in relation to individual expectations (Rutledge et al., 2014). However, it is not yet known whether animals are able to make predictions that can influence their future affective state (Mendl, 2023).

1.5 Theories about the emotions of animals: discrete (basic) and dimensional concepts

The conceptualisation of emotions as independent (discrete) entities can be considered as Darwin's first contribution to human understanding of the expression of emotions in animals (Figure 3; Darwin, 1872; Ekman, 2009).

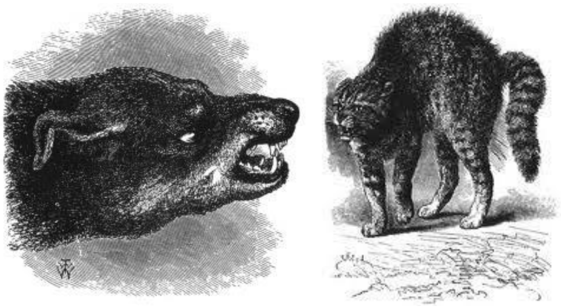

Figure 3. Expression of anger (snarling dog) and fear (cat terrified at a dog) in animals (Darwin, 1872)

In 1992, Ekman suggested that human beings have a basic set of discrete emotions, which are the result of different neural systems (Figure 4; Ekman, 1992).

Figure 4. Basic emotions in humans (Ekman, 1992). A= anger, B=surprise, C=disgust, D=sadness, E=happiness and F=fear

In animals, the discrete approach has found a great supporter in the psychologist and neuroscientist Jaak Panksepp, who has argued that discrete basic emotions such as anger, fear, panic, lust and care are generated by independent neurobehavioural systems (Panksepp, 1998). There was then a shift from the discrete to the dimensional approach, i.e., from the study of specific emotions to the study of their basic universal affective characteristics, namely valence, or degree of pleasantness, which can be positive or negative (neutral states are not considered emotional states), and arousal, or level of activation, which can be high or low. The dimensional theory of emotions has its origins in studies of human psychology and has been advocated by the psychologist James Russell, among others. In contrast to the discrete approach, the dimensional approach considers a single neurophysiological system (core affect) as the basis of affective states rather than, as mentioned above, multiple neural systems (Russell, 2003). In addition, according to this model, the human experience of emotion occurs through the combination of two or three evaluation dimensions, of which the two most used are valence and arousal. Subjective experiences can be represented in a two-dimensional space, with one dimension representing affective states (positive *vs* negative), and the other dimension representing the level of arousal (low *vs* high). The application of the dimensional model to non-human animals is due to Mendl et al. (2010), who adopted this type of emotion representation. An attempt to harmonise the discrete and dimensional approaches was made in 2010 by Mendl et al. by developing an integrated framework. According to this framework, in the two-dimensional representation of core affect, several discrete emotions fall into quadrants or basic states (Q1, Q2, Q3, and Q4) that identify a particular emotional valence and arousal (Figure 5). This can be explained by the fact that different discrete emotions are supported by different behavioural urges. Discrete emotions generated from core affect, stimuli and contextual evaluations also guide position and movement within the core affect itself, along with sensations and motivations. The location within the core affect depends on the individual's experience of success or failure in acquiring rewards or avoiding punishment, and there are biobehavioural systems that have evolved to perform these two important tasks (Mendl et al., 2010).

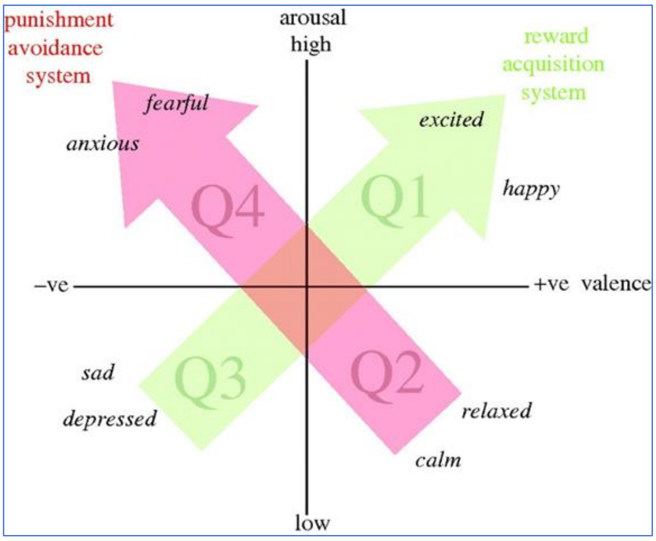

Figure 5. Core affect representation (from Mendl et al., 2010)

1.6 Emotions and animal welfare

1.6.1 How are emotions linked to animal welfare?

The importance of considering the affective states of animals when assessing their welfare was already highlighted in the Brambell Report: "*Any attempt to evaluate welfare [...] must take into account the scientific evidence available concerning the feelings of animals*" (Brambell, 1965); however, the relationship between these two concepts requires further discussion. There are currently two views: one sees affective states as equivalent to animal welfare (Bradshaw, 2019); the other sees them as a component of animal welfare (Kirkwood, 2007). Traditionally, animal welfare has focused on the avoidance or minimisation of negative states/affects, among which pain has received primary attention (Mellor, 2016). More recently, there has been a shift in the view of animal welfare from the absence of negative states/affects to also, if not predominantly, the presence of positive states/affects, such as pleasure, in the animal's life. The absence of pleasure or positive affect can therefore characterise a state of affective distress in the individual (Boissy et al., 2007). From this perspective, inputs, i.e. the factors that affect animal's life (e.g., available resources and management practices), should no longer be interpreted only as risk factors that have a negative impact on animal welfare (Figure 6), but also as benefits that have a positive impact and lead to an increase of the animals' well-being (EFSA Panel on Animal Health and Welfare, 2012). The old concept of animal welfare seen as absence of negative experiences (e.g., absence of pain, absence of fear, absence of disease) has been gradually integrated or even replaced by the concept of "positive welfare" (Mellor, 2016).

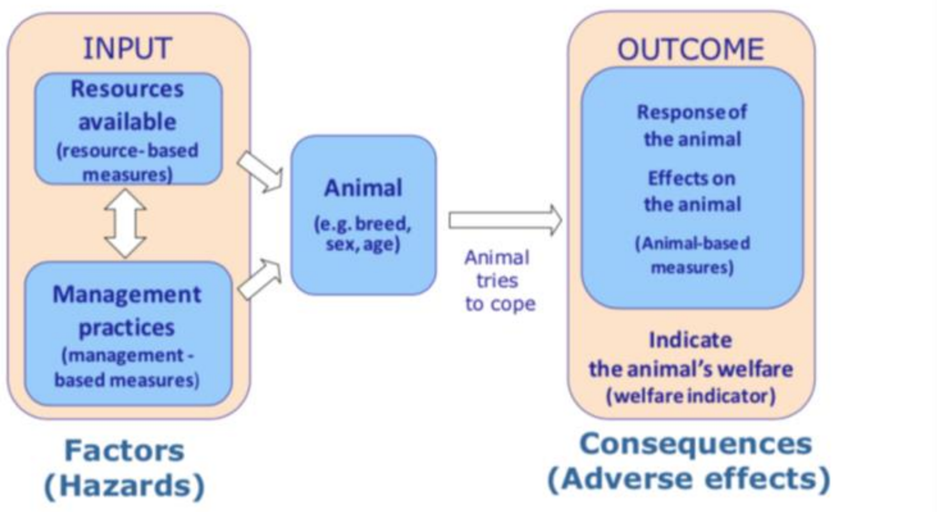

Figure 6. Diagram of risk factors (available resources and management practices) affecting the quality of animal's life. The outcomes of these negative factors are measured directly on the animal (animal-based measures; EFSA Panel on Animal Health and Welfare, 2012).

The term "positive affective engagement" was coined by Mellor in 2015 and refers to the ability of animals to experience positive affect when engaging in rewarding behaviours that have individual motivation behind them. Positive affective engagement represents one of the main features of positive welfare, along with the ability of animals to experience positive emotions and happiness, which determine their quality of life (Lawrence et al., 2019). Recently, Webb et al. (2019) proposed an attempt to structure the concept of animal happiness from the studies available in the literature on human quality of life and happiness. According to Webb et al. (2019), the balance between positive and negative affective states can be expressed as the animal's overall happiness and is, therefore, a long-term condition, thus differing by definition from the concept of emotion. The relationship between positive and negative affective states, i.e. “Affect balance”, is a focus of interest of the scientific community, and fostered the cost action 21124-LIFT: “Lifting farm animal lives – laying the foundations for positive animal welfare”. LIFT aims, among other things, to define the key concepts around positive animal welfare, to include them in the assessment of farm animal welfare. The welfare assessment protocols for small ruminants (AWIN protocols) include “positive emotional state” as a criterion for the assessment of appropriate behaviour. According to these protocols, the animals' ability to experience positive emotions can be assessed using only the Qualitative Behaviour Assessment (QBA), which will be described in sub-chapter 2.6.

1.6.2 The importance of a good Human-Animal Relationship (HAR)

The human-animal relationship (HAR) can be defined as the outcome of a dynamic process consisting of past interactions between humans and animals, which has a retroactive effect on their future interactions (Waiblinger et al., 2006). Emotional states arise as a result of a process of evaluation of humans or a context involving contact with humans by the animal, and are therefore directly involved in HAR (Désiré et al., 2002). The animal's perception of, and subsequent response to, humans occurs spontaneously or as a result of a process of associating the presence or behaviour of humans in general, or of someone in particular, with positive, neutral or negative consequences. The perception of and response to humans would therefore evoke emotions and motivations in animals in two directions: “positive/pleasant and negative/unpleasant”, which in turn would improve or worsen the quality of HAR (Waiblinger et al., 2006; Figure 7).

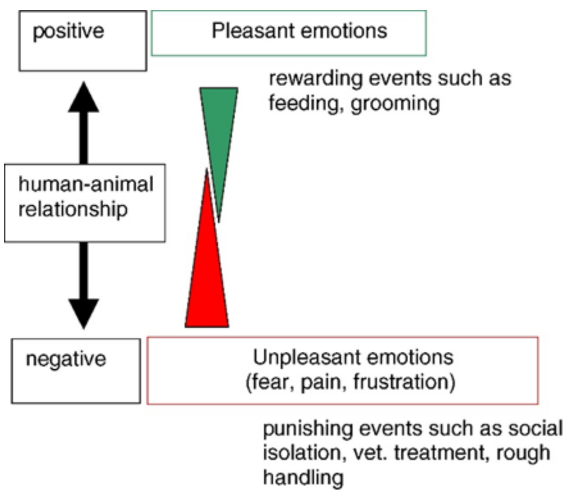

Figure 7. The two directions of emotional valence (unpleasant and pleasant) that influence the human-animal relationship (from Waiblinger et al., 2006).

Fear plays an important role among the emotions involved in an animal's response to humans, and its intensity varies with the quality of HAR. Factors involved in determining fear of humans include species and husbandry system, but also social attachment to humans, type and frequency of previous experience with humans and management quality (Waiblinger et al., 2006). Farmers/stockpeople therefore play an important role in determining the level of fear of farm animals.

In Italy, no specific legislation exists to protect the welfare of sheep and goats and only general rules are available, that apply to all farm animals, in the Legislative Decree 146/2001, which transposes Directive 98/58/EC. The annex provided for by art. 2, paragraph 1, letter b of this Legislative Decree is dedicated to the personnel, which must be present on each farm with "a sufficient number of employees with adequate professional skills, knowledge and competence".

The ability of animals to discriminate between humans underlies the possibility of being attracted to humans and developing a relationship with them (Waiblinger et al., 2006). Recent scientific studies show that goats are able to discriminate facial expressions (Nawroth et al., 2018) and emotional valence in human voices (Mason et al., 2024), and it seems that they are also able to discriminate familiar faces using a combination of visual and acoustic stimuli (Mason et al., 2023). In sheep, the ability to recognise familiar and unfamiliar human faces from two-dimensional images has been demontrated (Knolle et al., 2017), while Destrez et al. (2013) suggest that the positive long-term emotional previous experience with humans underlies the possibility of such discrimination.

Consistently with the shift that has been occurring from negative to positive welfare, also the assessment of the human-animal relationship should shift from responses reflecting negative HAR (i.e., avoidance of humans) to those reflecting positive HAR (i.e., approaching humans; Waiblinger et al., 2006). The AWIN welfare assessment protocols for goats and sheep (AWIN, 2015a; AWIN, 2015b) use the latency to the first contact test (for goats) and the familiar human approach test (for sheep) to assess the quality of the human-animal relationships. The Familiar Human Approach Test (FHAT) is an animal-based indicator that has been also tested and validated to assess the quality of the human-animal relationship in semi-extensive dairy goat farms (Battini et al., 2021; Battini et al., 2023). Interestingly, in the AWIN protocols and in their extensions to other farming systems the focus is on the positive rather than on the negative HAR, in contrast to, for example, the approach suggested for cattle in the Welfare Quality assessment protocol, which suggests using the Avoidance Distance Test (ADF) to assess the quality of the human-animal relationship (Welfare Quality® (2009). However, it would also be interesting to assess the intensity of the animals' attraction to humans, which could be measured by tests with familiar and unfamiliar humans (Waiblinger et al., 2006). However, current tests to assess HAR in goats involve either familiar or unfamiliar humans, but not a combination of the two (Celozzi et al., 2022), whereas in sheep only familiar humans are involved.

But why is a good human-animal relationship important, and how can it be improved by humans? The answer to this question is presented in a review that my research group and I published in 2022, entitled 'Humans and goats: Improving knowledge for a better relationship'. This review focuses on goat-human interactions, but some considerations, such as the possibility that humans provide an opportunity for goats to experience positive emotions, can be generalised to other species, such as sheep.

1.6.3 HUMANS AND GOATS: IMPROVING KNOWLEDGE FOR A BETTER RELATIONSHIP

Celozzi, S., Battini, M., Prato-Previde, E., & Mattiello, S. (2022)

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1. Introduction

The relationship between humans and goats has been dramatically changing during the last millennia, evolving from bare hunting for subsistence to intensive farming. This occurred through a long domestication process, initiated about 10,000 years ago, which led the still existing wild ancestor (*Capra aegagrus*) to adapt to farming conditions and to a close relationship with humans. Domestic goats (*Capra aegagrus hircus*) are now a widely used and increasingly economically important commercial species, reared in a variety of systems, going from very intensive systems to extensive pasture-based systems. Small holdings and family farms are also widespread all around the world, especially in rural communities in the mountain or in tropical and subtropical areas. Goat population numbers have been increasing in the last 30 years, doubling their consistence and reaching a total population of 1.09 milliard heads, most of which are located in Asia (51%) and Africa (43%) (data retrieved from FAOSTAT: https://www.fao.org/faostat/en/#data/QCL; last access on 20 January 2022). Therefore, goat farming plays an important role in the economy of many countries, and it deserves attention in order to make it efficient and profitable, as well as for ethical reasons.

Improving the human–animal relationship may represent an effective low-cost strategy to increase animal performances and welfare, with no need of high investments, and might therefore be implemented even in low-income countries, where goats are widespread. For this aim, a very good knowledge of the mechanisms underlying the formation and consolidation of this relationship is crucial.

Multiple interactions between individuals lay the foundations for a consolidated relationship between the parties: the predictable expectations determined by these interactions influence the nature and perception of future encounters [1]. Bonds can be formed between animals of the same or different species, but this review will only focus on the relationships that develop between humans and animals, particularly between humans and goats. The relationship resulting from progressive interactions between humans and animals is a dynamic process usually called the "Human–Animal Relationship" (HAR) [2] and is also defined as "the degree of relatedness or distance between the animal and the human" [3]. This relationship implies that animals are able to memorise interactions with humans: the recall of the type of interaction has a strong and long-lasting impact on animal welfare [1]. Thus, the establishment of this relationship implies a mutual recognition. However, many animal species can both generalise [4] and discriminate in their experience and recognition of humans (cows: [5], sheep: [6], goats: [7]). This ability is also true for the stockperson who can establish an individual relationship with a few animals and a general HAR with the herd [8]. According to [9], humans may be perceived by farm animals as predator, prey, part of the environment without social significance, symbiont and conspecific, even if these roles are not mutually exclusive and sometimes questionable (e.g., symbiont and conspecific) [3]. Furthermore, three categories of emotions can describe the impact of humans on animals: negative, neutral, positive emotions. Despite centuries of domestication, it is likely to assume that prey species, such as goats, may perceive humans as predators to be feared, although close and frequent contacts may change this perception [10].

Recently, in [11] updated the Five Domains Model modifying the name of the fourth Domain from Behaviour to Behavioural Interactions. The revision of this Domain reflects the conscious behavioural choices the animals make and their perceptions of external circumstances towards humans. Animals actively seek for contacts with people and positive interactions are rewarding for them, increasing the motivation to engage with humans. Hence, the improvement of HAR quality is an opportunity to enhance both welfare and affective states. The authors list some behaviours of humans in specific situations that can positively influence the animals' affective state, e.g., humans offering foods, the presence of humans providing feelings of safety, humans participating in enjoyable routine activities or calming animals in threatening circumstances [11]. In contrast, if the behaviour of humans prevents the animals from receiving such a pleasant reward (e.g., limited human contacts and/or actions directly unpleasant, association of the human presence during threatening circumstances), animals may experience negative affective states.

On the farm, the quality of the stockmanship has the greatest potential to determine the quality of HAR. The improvement of the relationship between stockpersons and goats leads to a number of benefits for both parties; conversely, a poor relationship leads to clear impairments. From the farmer's point of view, the quality of HAR influences the performance of animals and the ease of handling them, as well as the satisfaction for the work [12]. It is well known that poor HAR negatively affects the productivity of cows [13,14], pigs [15] and poultry [16]. To the best of our knowledge, only one study investigated the effect of HAR on goat milk production [17], showing an impaired ejection of alveolar milk in goats that had received few contacts with humans. The results of this study highlight how fear of humans negatively affects the productivity of goats, as fostered by [18]. However, the quality of HAR did not affect goat milk composition (e.g., content of fat or protein; [19]), probably due the chemical stability of goat milk that is only altered during diseases (e.g., [20,21]). Considering other studies on the effects of HAR on goats' productive performance, heavier weight gains were recorded in response to a greater possibility of contact with humans in feral goats kept in captivity [22]. In [19] also observed a significant increase in heart girth, possibly due to changes in appetite or nutrient uptake and energy expenditure in goats subjected to positive physical manipulation. Furthermore, in [23] showed that adverse manipulation might have negative consequences on placental morphology and foetal survival in pregnant goats. Interestingly, the same study also showed that human positive interactions with pregnant goats lead to a positive effect on maternal care and on the behaviour of their goat kids.

A relevant side effect of investing in the formation of a positive HAR concerns the consumer's perception of the sector [24]: high standards of animal welfare are an important societal issue, and there is the expectation that animals raised in production industries receive good care and are treated humanely [25]. The failure to meet these expectations diminishes public acceptance and trust, posing a threat to the "social licence" to farm (i.e., public acceptance and support), and in turn, leads the sector to economic losses. Hence, providing care and offering opportunities for the animals to enjoy positive experiences helps meeting consumers' expectations about the animals' rights to have a life worth living [26].

From the goat's point of view, besides being a reward for animals and a social enrichment, good HAR quality can balance the negative experiences the animals usually have in the farm (e.g., vaccinations, mutilations). Early and frequent social interactions with humans can help to reduce stress due to manipulation procedures [27], such as sperm collection by transrectal ultrasound-guided massage of accessory sex glands in goat bucks [28] and the transport handling phase [29]. Habitual interactions with humans can also contribute to the reduction of stress due to adaptation to a new environment such as a laboratory [30] or a new farming system [22].

Although no information is available on goats, a good HAR may positively influence stress resilience in farm animals (heifer: [31]). Positive contacts with humans can affect the physiological response to stress in animals, improving their immune response and thus, their resistance to diseases (poultry: [32]. However, at present there is a lack of studies concerning the effect of human contact on the health of other livestock species, including goats [33]. Alcedo et al. [34] suggested that positive human–animal interactions may be important in promoting goat health through gentle treatment during practices such as deworming or during situations where welfare is poor, such as disease, gestation and kidding, although these aspects were not directly investigated in this study. A good HAR can also have a positive effect on the long-term mood and cognitive abilities of goats, as observed in rescued goats subjected to gentle treatment for two years after having previously experienced a situation of poor welfare [35]. Similarly, in [36] highlighted how the type of experience lived with humans can influence the ability of goats to correctly interpret the visual signals of human beings and consequently goats' behaviour of choice and approach.

Furthermore, in [37] stressed that stockpeople must work on the quality of the rela- tionship with small ruminants (and thus also with goats), especially when they are not used to frequent handling, such as in the case of extensively reared meat breeds. This is one of the major concerns included by [38] in their evaluation of welfare issues in extensive production systems. Extensively reared livestock are subjected only to sporadic and seasonal encounters with humans, frequently associated to aversive situations (e.g., vaccination, herding for weaning). Based on the experience on cattle and sheep, the authors suggest strategies to mitigate stress and fear reactions in animals, such as the training of young animals to stockpeople moving horseback or using motorcycles, the avoidance of unnecessary force or noise, the provision of food during stressful events [38].

A good human–animal relationship can be useful in the scientific field, for example, when it is necessary to make behavioural observations, in order not to influence the results [39]. Cox et al. [40] also stressed the importance of proper care and management of animals in order to obtain valid data in scientific research.

The importance of assessing the quality of HAR is a fundamental part of on-farm welfare assessment protocols. This criterion has been addressed in the welfare assessment protocol for goats reared in intensive conditions developed by the EU-funded project "Animal Welfare indicators" (AWIN) [41].

The human–goat relationship can also be seen from a social and cultural point of view, which goes beyond goats' mere production role. For example, in some African populations, goats are rarely slaughtered to satisfy the need for food, and they are rather kept for other "intangible" reasons, such as building and sustaining reciprocal ties, gaining prestige and respect, or for rituals [42]. In more developed countries, goats can be seen as non-food producing animals also in educational programmes. For example, a good human–goat relationship can have beneficial effects on children's development and education. Loyd et al. [43] examined the attitudes of parents of middle school children in an urban county in the south-eastern United States towards the use of goats in a farm to school program. The study showed that parents had a positive perception of goats, as they stimulated the interest and enthusiasm of their children for school, increasing their sense of responsibility through the care of the animals and allowing them to spend more time outdoors. Similarly, in [44] highlighted how the experience of daily care of goats in a Tokyo elementary school contributed to a greater familiarity of children with these animals and to stimulate ideas on human–goat coexistence. Moreover, in [45] reported that the use of goats in Japanese schools stimulated children's interest and empathy towards these animals, an attitude of respect for living beings in general, a greater sense of responsibility and encouragement to learning. The goats also strongly stimulated the sense of collaboration of the children for taking care of these animals. In some cases, therapeutic effects of goats on children were found, such as the reduction of the frequency of problematic behaviours and of the reluctance to go to school. Goats also played a positive role in calming children's hurt feelings when something unpleasant happened at school.

In addition to the positive effect that goats had on children, contact with these animals also had positive repercussions for disabled people. In fact, multiply disabled persons established a positive social bond with goats, which was beneficial for increasing patients' attention, active participation and expression of joy [46].

In conclusion, human–goat interactions may have important impacts on animals for production purposes, and also on human beings for education, recreational and cultural purposes, and for ethical reasons. Therefore, the aim of this review is to present the state of the art of the existing knowledge of the mechanisms underlying the formation and consolidation of this relationship. In particular, we try to elucidate: (1) how humans and goats communicate; (2) which are the factors affecting human–goat interactions/relationship; (3) how we can measure the quality of this relationship. We conclude with recommendations on how to improve the quality of such relationship, based on the results of the previous sections.

2. Materials and methods

The scientific literature on human–goat relationship and communication published up until December 2021 was searched in Web of Science and Scopus electronic Databases. Only full-text articles in the English language (both English and American spelling) were included in the search. The reference population included goats of all ages and sexes, either domestic, feral or wild, under any condition (from intensive farming to free-ranging animals, including also goats in petting zoo and residential institutions for disabled people). Papers were searched in different subject areas concerning Agricultural Sciences, Agriculture Dairy Animal Sciences, Veterinary Sciences, Biological Sciences, Behavioural Sciences, Multidisciplinary Sciences, Ecology, Neurosciences, Zoology, Psychological Sciences, Interdisciplinary Social Sciences and Communication.

The keywords searched were goat$ AND human$ OR farmer$ OR stockperson$ OR stockpeople OR owner*; goat$ AND interaction$ OR communication$ OR relation* OR care. These keywords were combined in different ways.

This process led to a total number of 66 articles, excluding those which were present in both databases. Fifteen further articles were added via the citation method. The abstracts of 81 articles were subjected to a preliminary screening process and only papers answering to the following questions were retained:

(1) how do humans and goats communicate?

(2) which are the factors affecting human–goat relationship?

(3) how can we measure the quality of human–goat relationship?

Papers that did not answer to these questions (e.g., papers dealing with intra-specific communication or interspecific interactions not focusing on goats and humans) were considered non-relevant for the present review, and were therefore eliminated.

After this screening, 58 articles were retained for full-text reading (Figure 1) and imported in the reference manager and text editor Citavi®, where the structure of the chapters was created. All these articles focused on domestic goats, except for one that was on feral goats. No articles were retrieved on the interaction between humans and wild goats.

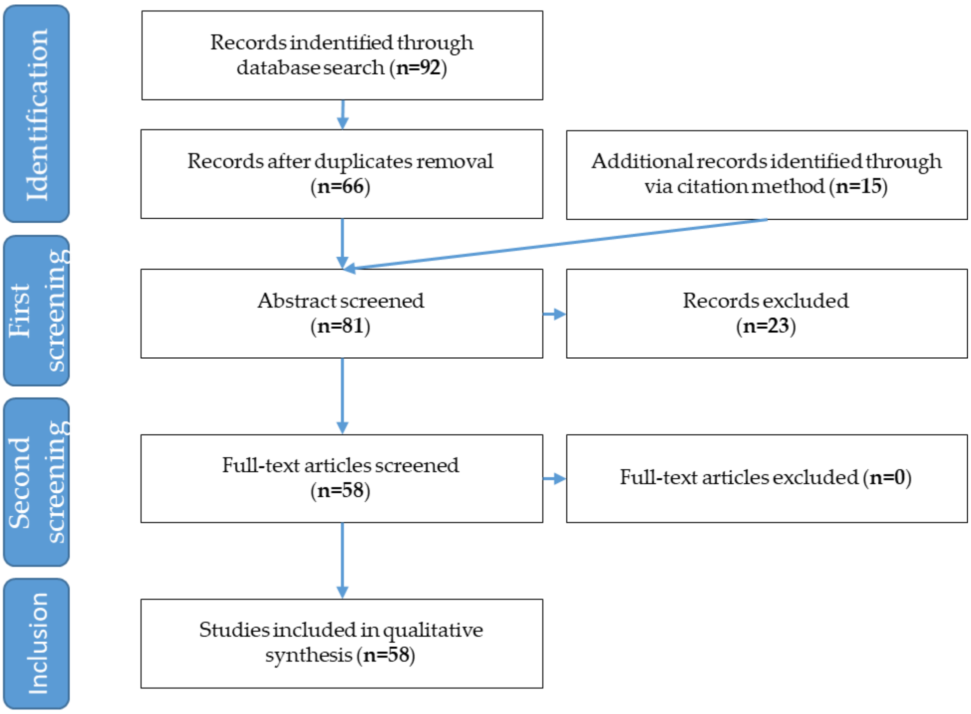

Figure 1. Flow chart of the systematic literature review process displaying exclusion and inclusion steps.

3. How Can Humans and Goats Communicate?

A fundamental assumption in order to establish a positive human–goat relationship lies in the human ability to understand signals emitted by goats and in the awareness of how goats react in response to human behaviour [47]. Therefore, communication between humans and animals influences the development of the HAR [2]. Furthermore, referential and intentional communication can provide cues which stimulate socio-cognitive processes [47].

It has been suggested that, during the domestication process, animals developed different levels of specialisation in the field of inter-specific communication with humans [48]. Compared to other species which have been domesticated mainly as companion animals, such as dogs and horses, goats, as with other farm animals, may be expected to have lower skills for communicating with humans [47], because selection during the domestication process has pointed mainly in the direction of decreasing emotional reactivity towards humans and increasing production traits, such as milk yield or weight gain, but not towards the development of a direct cooperation with humans [48]. Nonetheless, based on a study on the ability of goats to identify human cues, Kaminski et al. [49] hypothesized that the capacity of goats to react to human cues, even in the absence of specific training, might be a side-effect of the ancient domestication of this species. Recent research suggests that human–goat communication can also be seen in the frame of a broader posthumanist linguistic framework, and therefore, that interspecies interactions may be included within the traditional linguistic theory [50]. In fact, although goats do not use verbal communication, humans can speak to them and can learn to understand their minds, consequently adapting their behaviour towards the animals [45].

Communication, either intra- or inter-specific, plays a fundamental role in social relationships and has many functions, such as localizing and identifying other individuals, gathering information on food or shelter location, sending commands, establishing or strengthening the social status, establishing or maintaining social relationships or signalling a temporary physiological state (e.g., oestrus signals). Communication signals can be either visual (e.g., postures), acoustic (e.g., vocalizations), olfactory (e.g., emission of particular odorous substances, such as pheromones), gustative or tactile [51]. For the scope of the present review, only intentional communication signals between goats and humans (and vice versa) will be reviewed, and effective intentional communication signals retrieved from 23 relevant papers are summarized in Table 1.

Signal category[1]	Behaviour	Emitter	Receiver	Meaning/goal	Ref.
A	Acoustic signals at different tones	Human	Goat	Training to shape discrimination	[52]
A	Clicker sound previously associated with food reward	Human	Goat	Training to wear a halter	[53]
A	Loud vocalizations	Human	Pregnant goat	Negative handling treatment	[23]
A	Speaking	Human	Goat kids	Positive handling treatment	[54]
A	Speaking in a soft voice	Human	Pregnant goat	Positive handling treatment	[23]
A	Speaking in a soft voice	Human	Goat	Inviting goats to approach + positive handling treatment	[55]
A	Vocal call “Come here”	Human	Goat	Call for goat’s attention	[36]
A	Vocal call “Come here” or "Come on, honey"	Human	Goat kids	Call for goat’s attention	[50]
G	Licking	Goat	Human	Positive feelings, search for contact	[56]
O	Smelling	Goat	Human	Positive feelings, search for contact	[56]
T	Biting and pulling human’s clothes	Goat	Human	Negative feelings, discomfort	[56]
T	Contact alternation (frequency and latency)	Goat	Human	Asking for help to solve problem	[48]
T	Establishing physical contact (rubbing, nosing, pawing a hand or leg or jumping up)	Goat	Human	Asking for help to solve problem	[57]

T	Physical contact (latency and duration)	Goat	Human	Asking for help to solve problem	[58]
T	Pushing human’s arm and hands with head/horns	Goat	Human	Negative feelings, discomfort	[56]
T	Rubbing the head, placing it on human’s lap	Goat	Human	Positive feelings, search for contact	[56]
T	Brushing head and back	Human	Goat	Inducing changes of emotional state	[59]
T	Massage	Human	Goat	Promoting goats’ relaxation, improvement of HAR	[56]
T	Petting, scratching, stroking	Human	Goat	Positive handling treatment	[55]
T	Petting, stroking and scratching	Human	Pregnant goat	Positive handling treatment	[23]
T	Stroking	Human	Goat kids	Positive handling treatment	[54]
T	Stroking	Human	Goat	Positive handling treatment	[19]
T	Touching, stroking and brushing	Human	Goat	Establishing contact with goats	[46]
T/G	Chewing (contact of goat’s mouth with humans)	Goat	Human	Refusing to wear a halter	[53]
T/G	Nibbling	Goat	Human	Positive feelings, search for contact	[56]
T/G	Nibbling human clothes	Goat	Human	Two hypotheses: search for social contact or replacement behaviour in a poorly enriched environment?	[50]
V	Approaching	Goat	Human	Establishing contact with humans	[55]

V	Establishing visual contact	Goat	Human	Asking for help to solve problem	[57]
V	Gaze alternation (frequency and latency)	Goat	Human	Asking for help to solve problem	[48]
V	Gazing	Goat	Human	Searching for cues on hidden food	[60]
V	Moving away from the trainer	Goat	Human	Refusing to wear a halter	[53]
V	Moving toward the trainer	Goat	Human	Establishing contact with the trainer	[53]
V	Standing in front of the trainer	Goat	Human	Establishing contact with the trainer	[53]
V	Turning (90°) of goat's neck/head	Goat	Human	Refusing to wear a halter	[53]
V	Turning head and directing gaze away from the milk bottle	Goat kids	Human	Not interested in drinking milk	[50]
V	Body orientation	Human	Goat	Stimulating approach behaviour	[36]
V	Facial expressions	Human	Goat	Stimulating approach and interaction	[7]
V	Gazing	Human	Goat	Indicating a given direction	[61]
V	Head and body orientation	Human	Goat	Providing for cues on hidden food	[62]
V	Offering food	Human	Goat	Inviting goats to approach	[50]
V	Offering food (twigs)	Human	Goat	Inviting goats to approach	[46]

V	Open vs closed eyes	Human	Goat	Stimulating approach behaviour	[36]
V	Pointing the arm	Human	Goat	Providing cues on hidden food	[49]
V	Pointing the arm	Human	Goat	Providing cues on hidden food	[63,64]
V	Slow arm and hand movements	Human	Goat	Inviting goats to approach	[55]
V	Touching object	Human	Goat	Providing cues on hidden food	[63]
V	Touching object and moving the arm	Human	Goat	Providing cues on hidden food	[49]
V/A	Shacking a food container	Human	Goat	Attract goats' attention	[65]

[1] V: Visual signals; A: Acoustic signals; O: Olfactory signals; T: Tactile signals; G: Gustatory signals

3.1. Olfactory communication

Olfactory signals are particularly important for intra-specific communication in goats [66] and a wide range of odour signals is produced to send, for example, sexual (e.g., [67]) or maternal cues [68]. However, there is only very little evidence about the use of olfactory signals in human–goat communication. Only one of the articles included in the present review reports smelling behaviour, probably with the aim of obtaining olfactory cues, directed from goats towards a human being, which in this specific case was a person who periodically massaged the animals [56]. The reason why so few studies report information on olfactory communication between goats and humans is probably related to the common belief that humans' olfactory abilities are not particularly developed compared to those of other mammalian species. However, recent research aiming to compare human and animal olfactory capabilities demonstrate that this belief is not grounded on sound scientific data. In fact, in [69] suggests that humans have a well-developed sense of smell, which regulates a variety of human behaviours and that, in many cases, is more developed than that of several animal species. Therefore, the field of human–goat olfactory communication probably deserves further attention to gain scientific evidence about this communication means.

3.2 Visual communication

Visual communication can be used as a means for transmitting specific cues, and has therefore been often investigated in cognitive studies. Goats are able to correctly interpret visual cues from humans, such as pointing the arm towards a given direction, touching a given object or slowly moving arms and hands [49,55,63], even though the effectiveness of pointing the arm seems to be affected by the distance between the arm and the reward [64]. Body orientation is also an effective visual communication signal [36], whereas hand position (in front of or back the experimenter) and head orientation alone do not convey relevant information to goats [36,63]. However, human's head in combination with body oriented towards the goats seem to stimulate the attention of dwarf goats, that show active anticipatory behaviour for a reward (i.e., food), regardless of the fact that the experimenter's eyes are open or closed [62]. On the contrary, later studies [36] showed that open eyes may provide subtle cues to goats, which seem to prefer approaching experimenters with open rather than closed eyes. Other interesting effective cues are facial expressions: ref. [7] demonstrated that goats seem to prefer interacting with happy rather than with angry human faces. Contrasting results were found on the use of human gaze to communicate with goats: in fact, in [61] found that gazing was effective to indicate a direction to goats, whereas [49] did not report successful results on the use of human gaze to provide cues for locating a hidden reward.

A postural signal, often used also on farm to attract goats' attention, is the action of offering food, as reported by [50] in a petting farm or by [46] in a pet-therapy experiment. This signal was also used by [65] in a cognitive test, where the experimenter shacked a container with dry pasta to motivate the goat to walk towards the food. In this study, the noise produced by the food shacked into the container probably also acted as acoustic stimulus to attract the attention of the goat.

From the goats' side, in [60] observed that goats gaze at humans when they are searching for cues in order to get a reward, but they also noted that this behaviour was more pronounced when the experimenter was facing towards them rather than when he was oriented backwards. Gazing at humans was also observed in unsolvable task test studies, where goats alternatively looked at the experimenter and at the inaccessible food reward, as if they were looking for cues to solve the task [48,57]. In contrast, in [58] could not confirm an increase of gazing duration nor a decrease of latency to gaze in goats searching for help to reach a food reward.

Other reported visual signals include body postures, such as goat kids turning their head and directing their gaze away from the milk bottle that humans are using to feed them, in order to show their lack of interest to be milk-fed [50]. Similarly, in [53] describes a goat turning its neck/head and moving it 90° to the left, right, up or down, or moving away from the trainer, in order to show its refusal to wear a halter. On the contrary, in the same study, the goat stood in front of the trainer or moved toward him to establish a positive contact. Approaching a standing human was also performed by goats during a test in order to establish contact [55].

In conclusion, these findings demonstrate that goats can successfully interpret some physical cues and visual signals from humans, such as body posture and, to a less extent, head orientation, and they seem to be sensitive also to subtle cues such as open eyes and facial expressions. On the other side, goats send visual signals to humans to seek for help. Furthermore, it is worth noting that visual signals may be particularly effective in the communication between goats and children because, being goats a medium-sized species, their face is close to that of children, who can therefore better observe goats' facial expressions and easily interact with them [45].

3.3. Acoustic Communication

Goat are a highly social and vocal species, exhibit vocal plasticity in response to the social environment [70], can individually recognize conspecifics' vocalizations [71] and are able to distinguish the emotional valence of conspecifics' vocalizations [72]. Therefore, vocalizations can be good indicators of the sender's characteristics, such as identity, body size, age, hormonal status and affective states, and a large amount of studies have been carried out on intra-specific communication in goats (e.g., [26,70,72–75]).

Nonetheless, to our knowledge, no studies have specifically taken into account aspects related to inter-specific vocal communication between humans and goats or between goats and humans. However, some vocal signals directed from humans to goats have been described, such as the use of vocal calls like “Come here” or “Come on, honey” emitted by humans in order to attract a goat’s attention [36,50], or anecdotic sentences like “Hmm, that’s tasty huh?”, emitted to invite goats to receive food on a pet farm [50]). These examples support the hypothesis that humans tend to be rather anthropocentric in their relationship with goats, addressing the animals using human language [50]. The human voice has also been used in association with different handling treatments [23,54,55], although specific studies aiming to identify the role of the characteristics of the human’s voice on goats’ reactions are lacking and certainly deserve attention for future research.

A particular acoustic stimulus is represented by a bridge stimulus, such as the sound of a clicker, used during operant learning procedures in training programmes. For example, a clicker sound that had previously been associated with a food reward was successfully employed by [53] to train goats to wear a halter and to be led by the halter.

Similarly, the use of acoustic signals at different tones was used by [52] in association to a positive reinforcement (i.e., water): lower tones (440 Hz; 80 dB) were associated with the reward, whereas higher tones (980, 1039, 1166 Hz; 80 dB) were not. This acoustical secondary reinforcement was effective to convey information to the goats about the right shapes to choose in a test to get the reward.

Apart from the above-mentioned reports, no other inter-specific vocal signal was retrieved from the selected papers.

Nonetheless, from the existing literature on intra-specific vocal communication in goats, we may find useful cues to assist humans in interpreting goats’ emotions by listening to their vocalizations, even though these vocalizations are not intentionally human-directed. For example, vocalizations related to positive emotions, such as the expectation of a food reward, show a lower frequency modulation extent and a less pronounced fluctuation in fundamental frequency of both closed- and open-mouth bleats, whereas in situations of high arousal, calls present higher fundamental frequencies and higher energy distribution, with a more pronounced variation [76]. For example, alarm vocalizations consist of high- pitched sneezes [77]. Vocalization characteristics of farm species, including goats, have been recently reviewed by [78], showing that some kinds of vocalizations are related to specific contexts: in goats, for example, guttural noises (gobbles) and snorts are related to sexual behaviour and bleats play a role in maintaining social relationships inside the group, whereas open-mouth bleats are usually related to negative situations, such as pain, or in some cases, may also be emitted in positive contexts, such as when goats are waiting for a food reward.

Anecdotic information on the ability of humans, including children, to perceive differences in goats’ vocalizations is provided by [45], who report that, after the replacement of a goat, the

teacher and children of a primary school noted these differences in their goats kept for educational purposes, and "wondered what this meant" [45].
Given that goats are highly vocal animals, further studies on the ability of humans to recognize the meaning, valence and arousal of goats' vocalizations would greatly help to strengthen and improve human–animal relationship. In the future, it would also be interesting to develop methods, such as smartphone apps for simultaneous decoding of multiple vocal parameters, to automatically identify the meaning of goats' vocalizations [78].

3.4. Tactile Communication

Tactile interactions are frequently used by goats for intra-specific communication (e.g., grooming, fighting [66]), but they can also be observed in inter-specific communication between goats and humans, for example, when goats are facing an impossible task to solve, especially if they have previously established a positive HAR [48,57,58]. In these studies, goats have been observed to look for physical contact with humans, such as rubbing, nosing, pawing a hand or leg or jumping up, probably in order to ask for cues or help to solve the problem and reach the expected reward, as described above for visual signals. Mastellone et al. [57] considered this tactile approach as a socio-positive (or at least neutral) behaviour, since no antagonistic events were recorded in his study.
A positive tactile communication was also observed by [56] in goats submitted to a massage treatment, where the animals were observed to look for contact with the masseur by rubbing their head and placing it on human's lap, and also nibbling the masseur, although this last behaviour probably also involved a kind of gustative communication. Goats also exhibited some contact behaviours that were considered as having a negative valence, such as biting and pulling human's clothes or pushing the human's arm and hands with head or horns, which might be associated with a feeling of frustration possibly generated by an association between the masseur and the feeding period that induced the onset of aggressive behaviour [56]; similarly, chewing behaviour, consisting of the contact of the goat's mouth with a person or an object (other than food) on a person, was also considered as a negative reaction to a human being (trainer) trying to convince the goat to wear a halter [53]. It is possible that this behaviour may convey some information by means of gustative communication, besides tactile communication, although from this study we cannot draw any conclusion in this respect.
From the human's side, in [56] the masseur established a tactile communication with goats by means of a massage that was aimed at promoting goats' relaxation and improving the quality of HAR. Similarly, in [59] proposes that brushing the goats' head and back may have a positive effect on the animals' emotional state. Petting, stroking and scratching the goats were also effective for

achieving a positive handling treatment [19,23,54]. Tactile signals (touching, stroking, petting, scratching and brushing) were also used by disabled persons to establish a contact with goats used for a rehabilitation therapy [46], and by familiar and unfamiliar humans in a handling test [55], demonstrating that goats are sensitive to these kind of signals.

3.5. Gustative Communication

Very little information is currently available on the use of gustative signals in human–goat communication. Some evidence is reported in one article [56] where goats are described as licking and nibbling a human person, and this was interpreted by the authors as a positive search for contact with humans.

Anecdotal reports of nibbling of non-food items, including, for example, humans' clothes, have also been reported by [50], although their meaning and effectiveness are not clear: the authors interpreted them either as a mean to look for social interactions, or as a replacement behaviour performed by animals living in a poorly enriched environment.

4. Factors Affecting Human–Goat Relationship

The factors that can influence the quality of HAR can be traced back to both animals and humans. As far as animals are concerned, both environmental and individual factors may affect HAR. Examples of environmental factors are the type of farming system in which animals are raised [54,79], or the handling experiences that generate expectations on the interaction with humans [1]. As for individual factors, the animals' genetic makeup explains the differences in temperament and therefore in docility towards humans [80], while other individual goat characteristics, such as social rank, explain behavioural differences towards humans [81]. Furthermore, humans' characteristics, such as attitudes and empathy toward animals, should be taken into careful consideration, as they determine the behaviour of the stockpersons when working with the animals, and can therefore influence the quality of HAR [82]. Empathy, in particular, underpins attitudes towards animals and has a profound influence on animal fear towards humans, which in turn may affect their performance and welfare [33,82]. Other human characteristics that play an important role in this sense are represented by the propensity to work with animals, the knowledge and technical skills of farmers [82] and their commitment and job satisfaction [33]. Beaujouan et al. [83] also underlines that it is mandatory to consider the work context in which stockpeople operate, in order to fully understand how the HAR develops. The human factors which influence the human–goat relationship are described in the "human perspective" section of this chapter. All the factors identified through the 17 retrieved articles are summarised in Table 2.

Table 2. Factors affecting the human-goat relationship.

Factor type[1]	Factor	Effect on HAR	Ref.
E	Human rearing of goat kids	> time of kids in proximity with humans in goat-human encounter test < latency of kids to approach humans in goat-human encounter test < kids' flight distance in goat-human encounter test	[80]
E	Human rearing of goat kids	< kids' avoidance distance in AD[2] test	[84]
E	Human rearing of goat kids	> confidence of kids with humans > ease of management in adulthood	[85]
E	Human rearing of goat kids	< flight distance in encounter and choice test > time in proximity with humans in encounter and choice test	[54]
E	Human rearing of goat kids	< latency of adult goats to approach human in encounter test in the home pen > time of adult goats in proximity of humans in encounter test in the home pen	[17]
E	Frequent contacts with visitors (in zoo)	> human-directed behaviour in an impossible task paradigm	[57]
E	Frequent contact with humans (entering the goat pen twice/day and walking calmly among the goats for 20 minutes)	< flight speed of feral rangeland goats in a flight response test	[22]
E	Farming system (intensive *vs* semi-extensive)	< latency to the first contact in intensive farms	[86]
E	Gentling treatment (friendly talking, gentle touching, stroking and hand feeding twice daily, over two weeks)	Performing of alternation of gaze and contact towards the human being in an unsolvable task paradigm	[48]

E	Gentling treatment (stroking goats' back and neck with eye contact for 10 min for 24 days)	Quicker approach to the experimenter in latency test faster habituation to the presence of the experimenter	[19]
E	Proportion of negative interactions during milking (e.g. talking harshly, hitting and kicking the goats)	> avoidance behaviour in goats; < approach behaviour in goats	[87]
E	Small farm size, low goats/stockperson ratio	> % of acceptance and contact with human in AD^2 test	[88]
E	Presence of environmental enrichments (i.e. tractor tyres; heap of compacted earth)	> distance from the human experimenter in an handling test	[81]
I	Temperament	> latency to proximity and latency to contact in different test situations in "timid" goats	[17,80]
I	Breed	Boer vs Tswana, Nguni and Xhosa lob-heared genotype: > flight times during handling; < flight speed during handling; < vocalization score during handling; < crush score (CS)3 during handling	[89,90]
I	Breed	Saanen and Murciano-Granadina easier to handle than more rustic breeds (hypothesis)	[91]
I	Low social rank	> proximity to a stationary human; < time to get used to stress due to handling and restraint	[81]
I	High social rank	> distance from a stationary human; > time to get used to stress due to handling and restraint	[81]
H	Considering goats pleasant animals	> possibility of pain recognition in goats	[92]
H	Higher empathy	> positive attitudes in relation to goats and working with them	[92]

H	Being raised on a farm	< human consideration of the goat as a pleasant animal to work with, entertaining and intelligent	[92]
H	Stockperson’s gender	in women > ability to interpret and understand goats’ experience and find easier to work with goats	[92]
H	Belief in the importance of positive contact with goats (i.e. stroking)	> goats willingness to be touched	[93]

[1] E=environmental; I=individual; H=human
[2] AD=avoidance distance
[3] CS= crush score: behaviour of an animal assessed when put into a crush, using a 1 (calm) to 5 (combative) scale

4.1 Environmental factors

The social and physical environment are important factors, which act on the nature of HAR. The social environment is particularly important, as learning to distinguish acceptable from unacceptable social behaviours is part of the normal socialisation process and development of social skills [1], which occur mainly in young animals, especially during the sensitive period for the establishment of this relationship [54]. For these reasons, early handling experiences and early contact with humans have a particularly marked effect on HAR and, according to a number of studies, positive interactions between humans and goats should be established as soon as possible within the farm and the results are particularly effective if performed with newborn kids. In fact, as goats are a precocial species, kids are able to interact with humans since the beginning of their developmental stages, thus allowing an early establishment of social bonds [57]. It has to be underlined that the effect of early socialisation may persist throughout time: for example, in goats, the persistence of the effect of human contacts is supposed to range between 8 [54] and 25 months [17].

Five studies investigated the role of human rearing of goat kids, including early contact and manipulation, on HAR. The first study dates back to 1988 [80]. Lyons et al. [80] compared the behaviour of goat kid twins, in which one was raised by the dam and the other by humans, finding that different early experiences led to different behavioural expressions of temperament that tended to remain stable and persist over time until 30 weeks of age. In particular, human-reared kids exhibited a significantly higher amount of time in proximity with humans, a lower latency to approach them and a lower flight distance compared to dam-reared kids. [17] also noted that different previous experiences in terms of frequency of opportunities for human contacts led to different goats' reactiveness in encountering an unknown human being as a novel stimulus, in a home pen. In particular, multiparous goats raised by their mothers showed a greater human avoidance behaviour, as suggested by the longer latency to approach and lower time in proximity with humans compared to multiparous goats separated from their mother at birth and bucket-fed 2–3 times/day for 10 weeks by humans. This was also accompanied by a reduction of milk ejection in dam-reared goats. Similarly, a study by [84] showed that, five weeks after weaning, artificially-reared kids showed shorter avoidance distances from humans than dam-reared kids. Toinon et al. [84] therefore suggests that bucket feeding, accompanied by positive physical interactions (e.g., gentle stroking), allowed one to establish a good HAR. Finan [85] also noted that, in an artisanal farm in the United States, the isolation of goat kids from their mother and bottle feeding allowed developing a very strong relationship between caretakers and baby goats. This experience of caring for goat kids represented an opportunity for caretakers to interact with them by calling them by name, a name the kids learned. By learning to trust humans and to enjoy their presence, the kids became more friendly toward humans and, therefore, easier to manage in adulthood.

However, artificial feeding (i.e., feeding with a multi-nipple bucket) in itself is not enough to shorten kids' flight distance, even when milk is delivered by caretakers. In fact, Boivin and Braastad [54] found that these factors (artificial feeding provided by the caretakers) were effective for improving HAR only if goat kids had also previously been isolated from peers for a short period (i.e., 10 min/day for 10 days) and handled by humans. The authors argued that a short period of isolation and handling at an early age is sufficient to reduce kids' flight distance, at least up to 7.5 months of age.

In addition to the early interactions between humans and goats, the number and quality of these interactions is also important for the establishment of the human–animal relationship. This has been addressed in 5 articles. Mastellone et al. [57] highlighted how frequent interactions with humans in a zoo led to a higher number of human-direct behaviours in goats. In this study, the authors found that, in an impossible task paradigm, goats with a long history of socialization (i.e., presence of visitors in the home pen almost daily) performed a higher number of visual, tactile and approach behaviours directed towards humans, compared to goats accustomed to the simple routine care by the zookeeper. In particular, tactile behaviours (i.e., rubbing, nosing, pawing a hand or leg or jumping up) were significantly more frequent in the socialised group. Furthermore, Miller et al. [22] demonstrated that more frequent interactions with humans, represented by entering the goat enclosure daily and walking calmly, were effective in favouring the adaptation of feral rangeland goats to intensive farming systems. In fact, these frequent interactions led to less aggressive reactions and reduced flight responses compared to those recorded in feral rangeland goats under similar farming conditions, which had experienced a lower degree of interactions with humans. In line with these findings, Yoshida and Koda [58] found a shorter latency to the first contact in intensive farms compared to semi-extensive farms, probably because intensively reared goats are more accustomed to contacts with different people.

A gentling treatment (stroking goats' back and neck with eye contact for 10 min for 24 days) was applied by [19] on adult dairy goats, resulting in animals that approached the experimenter more quickly in a latency test, and which habituated faster to the presence of the experimenter. However, Langbein et al. [48] suggests that a gentling treatment (friendly talking, gentle touching, stroking and hand feeding) conducted for a limited period of time (twice daily, over two weeks) may not lead goats to interact more with humans. Interestingly, the authors highlight how standard husbandry care in a farm context may be sufficient to ensure that the goats show referential and intentional behaviours directed towards the human being (alternation of gaze and contact) in difficult contexts (i.e., unsolvable task paradigm).

The physical environment in which animals live may also have an impact on the quality of HAR. For example, a study conducted on 30 Italian farms showed that the farm size significantly affected the percentage of goats that accepted being gently stroked on the head for >3 s by an unfamiliar person during the AD test. This percentage was higher in small farms (<50 lactating goats) than in large farms (>100 lactating goats); the percentage of goats that accepted to enter in contact with the unfamiliar person, but withdrew within 3 s, was also higher in small farms, although differences were not statistically significant [88]. The apparently better relationship of the stockpersons with goats in small farms might be due to lower workload in these farms, with a more favourable ratio between the number of goats and both the number of permanent workers and the number of milkers. It is therefore possible that having more time to dedicate to each goat may favour the creation of a more positive HAR. However, these results were not confirmed by [91], who did not find significant differences in the latency to first contact test between small and large farms in Portugal, although the latency was unexpectedly lower in larger farms (but this was attributed to goats' individual traits, as explained in the next paragraph).

Miranda-de la Lama et al. [81] showed that the presence of environmental enrichments, represented by a raised feeding, physical barriers and elevated areas (i.e., two tractor tyres, a heap of compacted earth), significantly affected the behavioural and physiological responses of dairy goats to manipulation and restraint. In fact, goats in the enriched environment showed higher blood cortisol levels and remained further away from the experimenter, compared to control goats, probably due to the greater possibilities of modulating the distance from humans in this environment. In the enriched environment, episodes of aggression against the experimenter were also more frequently observed, the causes of which remain to be investigated. A higher frequency of defence behaviours was recorded in control goats, which opposed greater resistance to capture, probably because of the lack of elements for hiding in the pen.

4.2. Individual Traits

In addition to environmental factors, genetic traits may also affect the HAR, for example, due to individual differences in terms of temperament, which are relatively consistent over time. Temperament determines goats' attitude towards humans, and goats' behavioural reactions. For example, a significant correlation was found between goats' individual temperament (expressed as "timidity" score, where goats that were more tense, watchful, excitable and fearful of people, and less friendly toward people, had the higher scores) and the response of goats to a set of five behavioural tests, including latency to proximity and latency to contact to several test objects, including a human being. These behavioural differences seem to be related to different pituitary adrenal responses [17,80]. Genetic differences also exist, for example, among goat breeds. Molale et al. [89] observed that Tswana goats responded more negatively than Boer goats to human manipulation. In particular, differences in flight time and speed between the two breeds were attributed to differences in goats' temperament, with higher flight time (i.e., the time required for a goat to travel a distance of 4 m after being released from a crush pen) and flight speed in Tswana goats, which were then considered more fearful of humans than Boer goats. According to the authors, the calm temperament of Boer goats would make this breed easier to handle. In [90] also found a calmer temperament in Boer goats, as well as in their crossbreds, compared to more "combative" breeds, such as the Nguni and Xhosa lob-eared in a context of manipulative practices (blood sampling and rectal palpation). Notably, Nguni and Xhosa goats exhibited a higher flight speed, a higher vocalization score and a more pugnacious behaviour when put into a crush than Boer goats and crossbreds. Moreover, Nguni and Xhosa goats' flight time resulted shorter than those of the other breeds. Temperament differences are suggested to affect the HAR also in the study by [91], who hypothesise that the better HAR that they recorded in large farms may be due to the higher prevalence in these farms of breeds like Saanen and Murciano-Granadina, which are more docile and easier to handle compared to the more rustic breeds bred in smaller farms, which are known to be more suspicious.

Social rank may also affect goats' relationship with humans. Miranda-de la Lama et al. [81] observed that higher-ranking goats maintain a larger distance (half a meter more) from an experimenter positioned in the centre of a box for two minutes, compared to lower-ranking goats. The authors explain this behaviour based on the considerations of their previous study, according to which goats with different dominance profiles would face the human presence differently and use different social strategies [94]. They also suggest that low-ranking goats get used to the stress induced by handling and restraint earlier than high-ranking goats, due to a passive profile in these low-ranking animals [94]. Despite this, goats with a low-ranking position required more time to be captured, and showed a higher number of aggression episodes toward humans, the causes of which still have to be investigated [81].

4.3. Human Perspective

Human–domestic animal relationships are inevitably unequal, involving human management and control of animals, either companion or farm animals.

A growing number of studies carried out on farm species such as pigs, cattle, horses and even alpaca and llama show that the farmer's or stockperson's characteristics play an important role in the welfare of these animals: in particular, personality, attitudes, empathy levels, beliefs in animal mental capacities are reported to affect behaviour towards the animals, concern for animal welfare and decisions on housing and management [95–101]. In addition, a number of sociodemographic variables, such as gender, age and education level and previous experiences with animals affect the way in which animals are considered, treated and cared for (e.g., [102–105]). Hemsworth and Coleman [98] pointed out the importance of positive attitudes and empathy of stockpersons toward animals, even in intensive production systems, and their role in reducing fear and stress. In fact, the way in which farmers think about their animals and feel toward them appear to be associated with the way they behave towards them.

One central characteristic for building a positive human–animal relationship is empathy, which can be considered as the ability to understand, share and care about another individual emotional state [106,107]. Empathy toward animals is associated with empathy toward people [108], and with positive attitudes toward animals and their welfare [105,109]. For example, Kielland et al. [110] found that dairy cattle with fewer skin lesions belonged to farmers with higher levels of empathy toward animal pain. Similarly, Norring et al. [111] reported that empathic veterinarians scored cattle pain higher.

In addition, attitudes towards animals, broadly defined as psychological tendencies to evaluate a particular entity (e.g., other humans or animals) with some degree of favour or disfavour [112], contribute to determining animals' health, welfare, productivity and management by affecting farmers' behaviour.

Among negative emotions, the perception of pain is certainly of primary importance, and [113] observed that stockpeople who believe goats are pleasant animals are more likely to worry about the pain they feel. The study revealed that females, older farmers and those raised in a rural district turned to the veterinarian more frequently. The perception of goats' pain by humans was also negatively associated with living on a goat farm, having agriculture as the main income and having experience in recognizing cases of pain. However, the authors highlight how the therapeutic decisions made by individuals can be different depending on the individual's tendency to engage in helping behaviours. In another study, Muri et al. [92] explored empathy and attitudes towards goats in the Norwegian dairy goat industry and the influence of some demographic variables. The authors tested 260 Norwegian goat farmers using multi-item rating scales specifically developed to assess attitudes and empathy toward goats. They found that the three empathy factors that emerged in their study (i.e., Emotional contagion, Personal distress and Perspective taking) were significant predictors of attitudes towards goats: each empathy dimension was associated with a different attitude factor. In particular, farmers who obtained higher scores in the empathy dimension labelled as "Emotional contagion" (i.e., emotional response to interaction with goats) showed a more positive attitude towards how pleasurable it was working with goats. Farmers scoring higher on the "Perspective taking" scale (i.e., taking the perspective of others regarding their emotions about goats) had more positive attitudes towards the general characteristics of goats;

finally, farmers scoring higher on the "Personal distress" scale (i.e., self-oriented distress when observing goats in negative circumstances) were more positive towards the ease of working with goats.

Different dimensions of attitudes and empathy were associated with different demographic variables including gender, age, having grown up with a companion animal or a horse or in a rural district. Gender differences were observed in both the "Perspective taking" and "Personal distress" dimensions, with women scoring higher than men. Women also showed more positive attitudes in relation to the general characteristics of goats and the ease of working with them. Having a companion animal or a horse in childhood was positively associated with "Perspective taking", while education was negatively associated with "Perspective taking" and "Emotional contagion". Farmers having grown up on a farm were less in agreement that goats are pleasant animals to work with, fun and intelligent. Farmers' attitude towards goats, and in particular, their belief in the importance of positive contact with them (e.g., stroking), was found to be linked to the behaviour of the goats in a test in which the animals were restrained and an unknown person tried to touch their chin [93].

In evaluating the validity and feasibility of various tests for HAR evaluation in goats, Mersmann et al. [87] studied the attitudes and behaviours of farmers towards goats. These were found to be associated with the different avoidance and approach tests used, where a higher proportion of negative interactions during milking (i.e., harshly talking, hit and kick the goats during milking) were associated with higher avoidance and lower approach behaviours in goats.

5. How Can We Assess the Quality of Human–Goat Relationship?

Besides the number of reviews that investigated the importance of positive interactions between humans (e.g., farmer, stockperson, milker) and farm animals (e.g., [1,2]), it is argued that measuring the positive relationship is difficult in animals and that it is more difficult to measure positive rather than negative relationships [1]. To this aim, Rault et al. [1] listed potential indicators that can be used to evaluate the quality of the relationship between humans and farm animals (e.g., behavioural changes, physiological changes).

Tests and indicators usually measure the reacction that animals have in response to a human's presence. However, a number of confounding effects can affect the results; hence, caution should be made when inferring a positive or negative relationship, as the response of animals can be influenced by several factors. According to [1], the voluntary approach of animals towards humans is a sign of positive relationship or curiosity, but the absence of seeking this interaction is not necessarily a sign of a negative relationship. The interest towards humans may sometimes depend on the context (e.g., indoors or outdoors). The motivation to the stimuli can also determine the reaction of animals towards the human. For example, when performing tests during the feeding time, the interest that animals show towards humans is reported to be low in dairy cattle because of other activities occurring at the same time (e.g., feeding, but also competition). A further confounding effect is the evaluation of social animals performing tests in a test arena with isolated animals: the results of these tests should be carefully evaluated since stress due to handling, isolation or novel environment can conceal the reaction to humans due to stress [2]. The interactions with humans are also species-specific and, within each species, breeds and attitudes can also act as confounding factors, determining the choice of the most appropriate test: e.g., local breeds are more responsive to humans and animals reared in extensive systems may avoid humans more than those reared in intensive systems since they are less accustomed to the constant and close presence of the farmer [91]. This aspects stress the importance to develop measures specifically validated for a species or breed in a specific context and discourages from using measures tested for other species or under different circumstances without prior validating them for the specific context of intervention [114]. Therefore, one of the aims of this review was to present an overview of potential measures of HAR quality that have been specifically applied to goats in different contexts.

5.1. Behavioural Tests

Behavioural tests measure the reactions that goats show towards human beings. After the first reaction, namely, orientation response [1], goats can react to humans showing a withdrawal behaviour and avoiding contacts, approaching the assessor and seeking for interactions, or standing still. Most of the tests can be classified according to three main categories: (1) reactions to a stationary human, (2) reactions to a moving human and (3) responses to handling. The assessor that conducts the test can be either familiar (e.g., farmer, milker) or unfamiliar (e.g., researcher, veterinarian) to goats. The test setting can be the home pen or a test arena; tests can be performed isolating the goats from the familiar group or together with peers [2].

Table 3 summarizes the behavioural tests developed so far to evaluate the quality of human–goat relationship.

Table 3. Behavioural tests for evaluating the quality of human-goat relationship.

Category	Assessor behaviour	Assessor	Animal category	Test context	Social context	Procedure	Variables	Validity[1]	Ref.
Stationary human	Motionless	Not specified	Adult	Test environment	Isolation	Goat restraint in a starting zone for 45 s and released in the arena with an assessor standing	Latency of proximity with the human, duration in proximity (within 2 m), sections crossed, mean distance from the humans	Y	[80]
Stationary human	Motionless	Unfamiliar	Adult	Test environment	Audience	Goat placed 5 min in the arena, peers behind a fence, then assessor enters and stays still for 5 min. Heart rate recorded by telemetry.	Duration in contact with the human, number of vocalisations, heart rate.	Y	[115]
Stationary human (1); Moving human (2)	1) seated human standing still, but moving the hand; 2) human approaching	Familiar	Kids	Test environment	Isolation	Two phases. Seated human: goat left alone in arena for 1 min, assessor enters and stands still for 1.5 min, stroking the kid if approaching. Moving human: the assessor approaches and tries to pet kid for 1.5 min	Duration in proximity (<2 m), in contact with the human. Vocalisation, sections crossed	Y	[54]
Stationary human; Moving human	1) and 2) motionless; 3) human approaching	Unfamiliar	Adult/ Kids	Home environment, indoor	Group/ Isolation	Three phases. 1) assessor enters pen and stands still, 2) assessor moves back and forth along the front fence, 3) assessor tries to touch goats	Latencies to approach the human (<1 m) and to make contact, duration in proximity (stationary or moving)	Y	[17,116]
Moving human	Human approaching	Unfamiliar	Adult	Test environment	Isolation	Goat placed in a circular runway, assessor walks (0.5 steps/s) behind it for 3.5 min. Blood sampling taken 3 days before the test, immediately after and 3 days after	Mean flight distance, following, approach, avoidance, vocalisation, human contact, urination (plasma cortisol)	Y	[80]
Handling	Handling	Familiar	Adult	Home environment, indoor	Group	Goats milked twice daily for 21 days by two persons. Then, the same persons score each goats behaviour	Seven behavioural scales: excitable, tense, watchful, apprehensive, confident, friendly to humans, fearful of humans. Milk ejection.	Y	[17]

Stationary human	Motionless	Unfamiliar	Adult	Home environ ment, indoor	Group	Assessor enters the pen and walks to a pre-determined spot, marking a 1.5-radius semi-circumference and starts the stopwatch. Assessor stands motionless for 5 min, back against the wall	Latency to the first contact performed by the first goat, percentage of goats that nuzzled or touched any part of the assessor (continuously recorded and at 1 min-scan), percentage of goats that entered the semi-circumference around the assessor, at 1 min-scan	Y	[117]
Moving human	Human approaching	Unfamiliar	Adult	Home environ ment, indoor	Group	The assessor enters the pen and stands in front of a goat (randomly chosen) at a distance of 300 cm, then starts moving slowly towards the animal at a speed of one step/s, 60 cm/step and the arm lifted with an inclination of 45◦, the hand palm directed downwards, without looking into the animal's eyes, but looking at the muzzle. When the goat shows the first avoidance reaction (moving back-wards, turning or shaking its head), the assessor recorded the distance between the hand and the muzzle of the animal, with a resolution of 10 cm. If the animal can be touched by the assessor, the distance is 0, and this is also defined as "contact".	Mean avoidance distance (cm) of the goats tested in the pen, percentage of goats that can be touched by the assessor during the AD test	n.t.	[114]
Moving human	Human approaching	Unfamiliar	Adult	Home environ ment, indoor	Group	Same procedure as [114] but with a starting distance of 200 cm. If the animal can be touched by the assessor but immediately withdraws, this is recorded as "contact"; if, after the contact, the animal accepts gently stroking of the head for at least 3 s, this is recorded as "acceptance".	Mean avoidance distance (cm) of the goats tested in the pen, percentage of goats that nuzzle or touch the hand of assessor during the AD test, percentage of goats that accept gently stroking of the head by the assessor for at least 3 sec during the AD test, percentage of goats tested	Y	[117]
Stationary human	Motionless	Unfamiliar	Adult	Home environ	Group	Assessor enters the pen and walks to a pre-determined spot, possibly in the middle of the long side of the pen. Then starts the stopwatch	Latency to the first contact performed by the first goat	Y	[86,91,117,118]

				ment, indoor		and stands motionless for 5 min, back against the wall			
Moving human	Human approaching and/or calling	Familiar	Adult	Home environ ment, outdoor	Group	The farmer approaches the goats in the usual manner. The assessor (out of sight of the animals) records the reaction of goats toward the farmer.	Three possible reactions of goats are recorded: avoidance, contact, approach	n.t.	[119]
Moving human	Human approaching and/or calling	Familiar	Adult	Home environ ment, outdoor	Group	The closest distance (m) of approach the group, before a flight response is evoked, is recorded. If an animal stands motionless, this is recorded as 0 m. Animals that voluntary approach the farmer and/or interact (sniffing or touching) are also recorded.	Mean avoidance distance (cm), percentage of animals voluntary seeking for human contacts	n.t.	[120]
Handling	Handling	Familiar	Adult	Home environ ment, indoor	Group	Unaware of being tested, the stockperson approaches and marks individual pre-selected goats on the head with a marking crayon, while an assessor evaluate his/her behavioural style, as well as the goats' behavioural responses during the procedure	Behavioural responses registered on five-point rating scales (1 = positive interactions; 5 = negative interactions)	Y	[93]
Stationary human	Human standing still, moving the hand	Unfamiliar	Adult	Home environ ment, indoor	Group	Chin contact test - The assessor stands in front of each goat, reaches out an arm with the palm pointing upwards, and gently moves the hand towards the goat's chin.	The goat's response to the hand is registered on a three-point scale: full acceptance, brief touch, full avoidance	Y	[93]
Moving human	Human approaching	Unfamiliar /familiar	Adult	Test environ ment	Group	3-min human approach test conducted after first- and seventh-handling experience of goats. Three main categories of reactions: 1) spatial (close, middle, far), 2) orientation (facing vs. turned-away), 3) structural (lie, stand, and nutritive and non-nutritive oral behaviours).	Percentage of duration of behaviour outcomes to create an approach index (AI): great approach (≥75% quartile), moderate approach (25% to 75% quartiles), least approach (≤ 25%)	n.t.	[121]
Moving human	Human walking along the feeding alley	Unfamiliar	Adult	Home environ ment, indoor	Group	Avoidance test at the feeding place - The assessor walks on the feeding alley with 0.5 steps/s, in a distance of about 80 cm parallel to	Percentage of animals still feeding when the assessor passes by	Y	[87]

						the feed barrier, assessing the reaction of feeding goats as the assessor passes by,			
Moving human	Human approaching	Unfamiliar	Adult	Home environ ment, indoor	Group	Avoidance distance test at the feeding place - From a distance of 200 cm, the assessor approaches individual animals than stand at the feeding place, constantly walking (speed of 0.5/s, steps of about 30–40 cm) with one arm 45◦ in front of the body, fingertips pointing to the ground and back of the hand towards the goat, until the goat withdrew or until touching. In case the goat can be touched but withdraws within 2 s an avoidance distance of 1 cm is assigned. Only when a goat accepts being stroked for more than 2 s an avoidance distance of 0 cm is assigned	Median value of avoidance distance at the feeding place, percentage of animals possible to stroke, percentage of animals with an avoidance distance greater than 1 m	Y	[87]
Stationary human	Motionless	Unfamiliar	Adult	Home environ ment, indoor	Group	Approach test in the pen - The assessor enters the pen and after a 30-34 s pause walks to a pre-decided testing place in the pen and marks three positions in a radius of 3 m. Then, the assessor stands 15 min motionless with the back to a wall.	Absolute number of goats into physical contact with the assessor, latency of the first animal touching the assessor (1-min scan), average number of goats within the 3 m radius (1-min scan), proportion of goats within 0.5 m to the assessor during the first 5 min	N	[87]
Moving human	Human approaching	Unfamiliar	Adult	Home environ ment, indoor	Group	Avoidance test in the pen - Two successive phases. Phase 1: the assessor walks for 1-2 min through the pen and observes the distance of the goats being closest to him/her. Phase 2: after leaving the pen for at least 2 min, the assessor re-enters the pen and approaches single animals, trying to touch them	Phase 1: estimation of the average distance from the group over the whole time. Phase 2: percentage of animals that can be touched	Y	[87]
Moving human	Human approaching	Familiar	Adult	Test environ ment	Group	Starting from a distance of 20 m, the assessor approaches the goats at a slow walking speed (1.5 m/s). When the flight response is induced,	Distance that the assessor approaches the group of goats at the time that all the goats run past,	N	[22]

						the assessor stops still after all the goats have run past.	average speed at which the goats run past and away from the assessor		
Stationary human	Motionless	Unfamiliar	Adult	Test environ ment	Isolation	The assessor keeps the eyes on the goat without moving the face or body for 5 min.	Behaviours: gazing, proximity (within 50 cm), contacting (at a distance of 1 to 10 cm)	n.t.	[58]
Moving human	Human approaching	Unfamiliar	Adult	Test environ ment	Isolation	The assessor approaches a goat leashed (1-m rope) on the side of the paddock, walking obliquely at a pace of 1 step/sec. If the goat remains stationary within 1.5 m, the assessor slowly moves the hand close to the face of the goat. If the goat does not escape and tries to smell the hand, the assessor tries to touch the goat's neck.	Scores (1 to 4): 1) goat moves away from the assessor (>1.5 m range), 2) goat stands still when the assessor is within 1.5 m range, 3) goat sniffs the assessor's hand, 4) the assessor touches goat's neck	n.t.	[58]
Stationary human	Motionless	Unfamiliar	Adult	Home environ ment, indoor	Group	The assessor moves to approximately the middle of the pen and begins timing the latency for each animal to approach within 60 cm. This measurement is capped at 10 min regardless of whether or not the animal approaches.	Latency to approach	n.t.	[19]

[1] Y=yes; N=no; n.t.=not tested

Eighteen papers were extracted during the literature review, with a total of 28 tests identified. Results equally split in tests that involve assessors moving in the pen, usually approaching individual animals, and stationary tests, with the assessor motionless in the environment with goats (both 39.28%). Only one stationary test requires that the assessor moves the hand to touch the animals [93]. Only two tests can be classified as handling procedures [17,93]. Both these last tests do not really imply an experimental handling, but the judgement or self-evaluation of the behaviour of goats during milking; hence, the handling refers to the way the stockperson behaves, touches and interacts with the animals. The remaining tests are classified as both stationary and moving human as they are normally composed of sequential phases [17,116]. The evaluation of the quality of the human–goat relationship has commonly been performed by unfamiliar assessors (67.86% of the tests) and preferably in the home environment (67.86%). The last attribute of the tests is the social context: 71.42% of tests have been performed with goats in their familiar group, even if some measures can be collected on individual animals. Only in one experiment, goats were individually tested but with peers in an adjacent pen with an open wire fence [115].

The absolute number of tests identified during the literature review does not correspond to unique tests. Indeed, many papers describe the same test with only little rewording of the description of the test or few changes. For example, [88,91,118] included a simplified version (e.g., less variables collected) of the latency to the first contact test validated by [117]. In turn, the same authors [117] applied a shorter version of the test developed by [19] (the test proposed by [117] is capped after 5 min if no contacts with goats occur, whereas the original test [19] is capped after 10 min). Before these recent studies, to our knowledge, behavioural tests measuring the latency to the first contact both in adult goats and kids were originally developed by [17,80,116]. Mersemann et al. [87] also collected the latency to the first contact, but the period the assessor remains motionless in the pen was increased to 15 min. Where tested for validity (Table 3), latency to the first contact test resulted valid, except for [87]. The duration of the test, whether capped after 5 or 10 min, depends mainly on the need to have feasible indicators, but it does not alter the validity of the test. When [117] validated this test, a large difference between farms labelled as "good HAR" vs. farms labelled as "poor HAR" was found (good: 8.0±4.7 s; poor: 136.0 ±55.2 s). The latency to the first contact test is generally reported as feasible: no specific training is required and time devoted to this procedure is limited. Furthermore, this test rarely induces fear or stress in animals [117]. The only suggestion made by the authors is to perform this test without the presence of bucks when evaluating the quality of HAR in dairy goats [117], because, in different trials, bucks were found to be the first to approach the assessor with females standing still in the pen. After removing the male, the authors found that the behaviour of females changed and approached the assessor. Mersmann et al. [87] also found that hornless goats show shorter latency compared to horned goats, probably because they respect the social distances among goats less than the latter and more easily make their way through the group to reach the assessor. After having been tested on farm, the latency to first contact test was included in the AWIN welfare assessment protocol for goats [41,88,91,122]. Based on the results of these preliminary on-farm tests, the AWIN protocol also contains indications that, if more than 24 s elapse before any contact occurs between goat and assessor, the HAR should be considered sub-optimal [41].

Tests belonging to the moving human category are largely represented by avoidance distance tests performed in the pen on individual animals in the group with peers. However, again, individual animals were approached using with similar procedures [41,91,114,117]. Differences are mainly related to the starting distance in front of individual goats to begin the test: this is 300 cm in [114], similarly to tests performed in dairy cows, whereas it is reduced to 200 cm in [41,91,117]. A larger starting distance (20 m) is proposed by [22], but this decision is justified by the objective of the assessment: the research investigated the habituation of feral rangeland goats to intensive farming systems, whereas [41,91,114,117] studied the reaction of dairy goats in commercial indoor farms. Even if the validity of the avoidance distance test was confirmed by [117], its application in large groups is likely not feasible, and many authors do not support its use, especially in on-farm situations (e.g., time consuming, extensive training required [117]). According to [117], the avoidance distance test is less suitable for goats than for cows, because of the different management. Dairy cows are accustomed to humans frequently entering the pen for cleaning operations, whereas goats are unlikely to experience close contacts, since the housing on deep bedding mainly involves the farmer topping up the pen with straw, frequently from outside, and rarely entering. During the testing of the prototype of the AWIN welfare assessment protocol for goats in Portugal, Can et al. [91] found that the feasibility and also the result of the avoidance distance test can be influenced by breed (e.g., local breeds appear to be more reactive) and production system (e.g., intensive vs. semi-intensive). The results obtained by the authors do not allow understanding if the test is not suitable for certain breeds or husbandry systems (or an interaction between these two aspects) or if the differences found are the result of a lower familiarity with humans in local breeds. Contrary to the experience reported by [114], Muri et al. [93] could not apply the avoidance distance test in the pen, as goats flocked around the assessor, making the evaluation impossible. Mersmann et al. [87] reinforced this assumption and added that this method is hard to be used if many shy animals are present, because they behave as a whole group, making it unfeasible to perform an individual assessment. Furthermore, the individual identification to avoid retesting the same animal is difficult, since goats usually have small ear tags with small identification codes or numbers. Can et al. [123] also found low consistency over time (from winter to summer, approximately four months between the two assessments). Mersmann et al. [87] tested and validated an avoidance distance test in the pen with dairy goats with two successive step. In the first step, the assessor only records the average distance of the group, considering the closest goat. In the second test, the assessor approaches individual goats and tries to touch them. The authors report that this test is feasible, not affected by external factors, and clearly related to the quality of the previous interactions between humans and goats (e.g., frequencies of contacts).

Moving human tests can also be performed at the feeding rack. Mersmann et al. [87] tested two procedures: the first involves the assessor walking along the feeding alley, recording the number of animals that continue feeding when the assessor passes by. The second resembles the avoidance distance test at the feeding rack already used in cattle, with the assessor approaching individual goats during feeding and measuring the individual avoidance distance. The authors reported that the feasibility of both avoidance tests at the feeding rack is supposed to be high (e.g., only 9 min for the assessor to pass by the feeding alley and record the number of feeding animals). However, due to some constraints (e.g., design of the feeding alley, presence of feeding belts), those tests were only applicable in the 70% of farms [87]. Some refinements could be made to support the use of the test involving the assessor walking on the feeding alley, as this test well describes the quality of the relationship with humans and, contrary to the findings in dairy cows [124], is not affected by confounding factors.

Muri et al. [93] applied a test that is classified as stationary human since the assessor does not walk towards goats, but stands still in front of individual goats and tries to touch the chin of the animal. However, reaching a goat to begin the test implies a sort of approach that can elicit a fear reaction, increasing the flight distance. Indeed, Muri et al. [93] reports that this test was only performed in half of the 30 farms evaluated.

Most of the tests were validated indoors, sometimes in a test environment, but also in the home pen. External validity was also confirmed in some cases where the application of test was made in commercial farms [87]. However, information on the use of behavioural tests to measure the quality of HAR in outdoors and in extensive husbandry systems is scarce. It is known that a reduced number of contacts during the whole year or in a limited period (e.g., summer grazing) can affect the perception and familiarity that animals have with humans (e.g., dairy cows after summer pastures show greater avoidance distance than before the grazing period [125]). The Familiar Human Approach Test (FHAT) included in the AWIN welfare assessment protocol for sheep [126] was used by [119,120] to evaluate the human–goat relationship in extensive systems. This test requires that the farmer behaves in the normal way, gathering the animals for inspection, and an assessor evaluates the behaviour of goats and the distance from the assessor. This test has never been validated in sheep [127] and has not yet been validated for goats either. However, Battini et al. [119] report that the execution of the test is feasible and reliable among assessors; hence, it is a promising indicator to be included in a welfare assessment protocol for goats in extensive farming systems, after its validation. Muri et al. [93] applied a similar test indoors: the farmer was asked to select 20 goats and to mark them as a part of the usual routine. Then, the assessor evaluated both human and goat behaviour on a five-point scale. This test provided interesting results; however, its repetition may yield biased results, because the farmer's behaviour may be affected by knowing that his/her behaviour is being observed and scored during the test [93].

5.2. Other Behavioural and Physiological Indicators

In addition to behavioural tests, other indicators have been used to measure the quality of HAR. Most of the indicators presented in this section are not feasible in on-farm situations but can be included in experimental settings.

Information that vocalisations provide about animals' emotions has been gathering increasing attention and has been addressed in recent studies in different species, including goats [78]. Goats may bleat with open and closed mouth. According to the limited research conducted so far, it is known that open-mouth bleats can be produced during both positive (e.g., anticipation of food) and negative (e.g., pain) situations, and are used to maintain social contacts with peers. A reduction in distress vocalizations, supported by increased proximity to humans, was interpreted by [54,115] as a positive perception of human presence. However, none of these studies provides information on the underlying emotions, but only changes in rate of occurrence of vocalisations. Goats also produce alert sounds. Alarm snorts, also called sneezes by [117], are described by [66] as a loud, high pitched, short and abrupt closed-mouth sound that goats produce to warn the group of a possible danger. Farmers are aware of the meaning of this sound and fostered research on this topic. Therefore, Battini et al. [117] tried to test the validity of this indicator, but unfortunately, the frequency of occurrence of sneezes in their study was too low to allow drawing any conclusion in this respect.

Among physiological parameters, oxytocin can be related to the perception that animals have of humans. Positive interactions with familiar humans are known to raise oxytocin concentration [1]. Oxytocin concentrations can be measured from saliva samples, which present the advantage that they can be easily collected in a non-invasive way; however, cost constraints for further analysis reduce the feasibility of this method in the on-farm situation, and make its use more suitable for research purposes [128]. Nevertheless, Lürzel et al. [55] could not confirm the validity of this indicator in goats, because they found a negative association between oxytocin concentration and animals being stroked by a human. The result is surprising, since goats freely chose to approach the human, but probably they did not perceive the interaction as positive. Some authors suggest that the situation, more than the action itself, may trigger the release of oxytocin [129]. Lürzel et al. [55] speculate that their unexpected results may be determined by the fact that goats were separated from their kids to perform the test, and were therefore experiencing a negative emotional condition, or by the physiological state of the animals (e.g., lactation). The low sample size of this experiment (n = 9) might also be responsible for these unexpected results.

5.3. Attitudinal Questionnaires

Attitudinal questionnaires developed for stockpeople may be valuable in predicting their behaviour towards animals, providing useful information on the quality of HAR, and validated questionnaires have been used in the egg, dairy, pork and veal industries (reviewed by [82]).

Compared to tests aimed at assessing stockperson behaviour, attitude questionnaires devised for different farm species are easier to standardise and to deliver in a standardised manner and can be used to compare farming situations and studies. In addition, attitude questionnaires can be combined with direct assessments of animal welfare (e.g., [130]). However, it is worth noting that there is still a need for harmonization of attitudes assessments in research [99]. Attitudes can be assessed using either qualitative or quantitative methods or integrating them to obtain a better understanding of farmers' attitudes towards animals and other aspects linked to animal welfare (e.g., [130]). A common approach used to investigate attitudes is to provide farmers with statements related to the object to be evaluated (e.g., dairy goats, adopting certain farm management practices, etc.), asking them to express their level of agreement on a Likert or semantic differential scale, and then inferring their attitudes by how they respond. Effects of interventions aimed at changing farmers' attitudes can also be assessed [131].

Attitudes toward animals result to be linked to empathy toward animals [98]: however, empathy has been poorly investigated in stockpeople [92,97,110]. For example, based on previous research, Hanna et al. [97] created their own questionnaire to measure attitudes and empathy toward cows. Kielland et al. [110], in order to assess farmers' empathy towards animals, developed and validated a photo-based pain assessment instrument depicting various conditions in cattle that could be associated with some degree of pain (PAI). As far as goats are concerned, Muri et al. [92] investigated attitudes and empathy in the goat industry involving a total of 260 dairy goat farmers. To our knowledge, this the first study assessing goat-oriented attitudes and empathy of stockpeople by means of questionnaires (web- and paper-based). In the study the attitude scale was based on the statements used by [13], while the empathy scale was an adaptation of the Animal Empathy Scale (AES) developed by [108]. Stockpeople behaviour was investigated in relation with work experience, other life experiences (e.g., being pet owner during childhood), demographic (e.g., age, gender) and educational level characteristics. This study reports that different dimensions of goat-oriented attitudes and empathy are associated with different demographic variables. It also suggests that the relationship between empathy and attitudes is complex and provides useful information for improving the methods for measuring animal oriented empathy in stockmanship [92].

Muri et al. [93] included a revised version of the previous questionnaire [92] during the development and testing of a welfare assessment protocol and stated that this tool has the potential to predict some welfare outcomes. For example, the authors found that, if farmers declared that they individually named most of their goats, the animals in those farms were calmer and less fearful [93]. During the validation of indicators of HAR, in [87,117] used a questionnaire originally developed for dairy cows to gather information about the attitude, empathy and behaviour of the stockperson towards goats, with researchers interviewing farmers directly on farm. Battini et al. [117] found that the predictive validity of the questionnaire is limited. It is argued that answers provided by farmers do not always correspond to their actual behaviour for a number of unclear reasons: the most likely is a conscious perception of social norms for which the farmer may respond dishonestly (i.e., it is unlikely that a farmer answers that beating or shouting at animals is a common practice). Presumably, anonymous questionnaires could provide more reliable answers.

6. Conclusions: Improving the Human–Goat Relationship

In several countries, animal farming has changed considerably and continues to change together with people's attitudes towards farm animals and concern for their welfare and humane treatment [24,26]. Average farm size has been increasing, and farming is becoming more mechanized, resulting in the reduction of human–animal interactions and a lowered attention towards individual animals; this leads to growing difficulties in detecting abnormal behaviour and illnesses in animals [132] coupled with increasing animals' fear of humans [133]. Conversely, animal welfare is now a relevant issue in many societies, and citizens' and consumers' attitudes towards farming, especially intensive farming, are changed, with a growing concern about animal welfare on farms [25].

Although animal welfare is a complex and multidimensional concept which allows for different definitions [134,135] and can be addressed differently depending on the actors involved (farmers, veterinarians, consumers), there is consensus among researchers, but also farmers, that HAR quality is relevant in all farming contexts in order to guarantee appropriate levels of animal welfare, and needs to be strongly encouraged and closely monitored, focusing on both the behaviour of the animals towards the stockpersons and the behaviour of the stockpersons towards the animals [18,136].

As noted by [137], farm animal welfare, and thus goat welfare, is not only a major concern for society and food production, but also represents an ethical issue. To improve goat welfare through positive human–goat interactions, it is essential to understand how goats communicate with humans, to gain knowledge on their behavioural and cognitive needs and capacities, and to promote and enhance this knowledge, especially in goat farmers, both at the theoretical and the practical level.

Species-specific studies on human–goat communication highlight that effective human–goat communication may take place by means of visual, tactile and auditory stimuli and, to a less extent, via olfactory and gustative stimuli, although further research may be useful to complement this knowledge. Spreading the information about this interspecific communication means among stockpersons and people having contacts with goats may greatly help in improving the human–goat relationship and may also facilitate all handling and management procedures with animals. This will also be facilitated by spreading information about recent studies on farm animals' socio-cognitive and affective capacities, which may have relevant implications for farm animal welfare and management [137–141]. Research on goats' cognitive abilities suggests that goats have an understanding of their physical and social environment and a number of socio-cognitive abilities that affect their interactions with conspecifics and humans [47,137]. For example, goats can differentiate among conspecifics using visual and/or acoustic cues [70,142,143], and they can successfully learn from conspecifics or even from humans [60,62] and use cues provided by conspecifics or humans [49,63]. They can also differ in their anticipatory behaviour depending on an experimenter's attentive state [62,63] and they are sensitive to human facial expressions [7]. This suggests that goats can adapt their behaviour based on the surrounding social environment. Therefore, it is extremely important that humans are aware of goats' reactions and adaptive behaviour, and act in a way that can help goats to feel at ease.

Based on the evidence coming from comparative psychology, human–goat interactions and relationships could be improved by bridging scientific evidence on goats' behaviour, cognition and emotions with practice: scientific knowledge needs to be shared with goat farmers in a functional and targeted way to enhance their capacity to correctly interpret goats' intraspecific and human-directed behaviours and signals, thus favouring them in doing their job at best. Increasing farmers' knowledge about goats' behaviour, communication abilities, cognitive and emotional processes could help improving their housing and management conditions and could be used to evaluate the use and treatment of animals during production. Moreover, the enhancement of farmers' knowledge and awareness that goats have cognitive abilities, experience positive and negative emotions and feel pain would be a useful prompt to perceive goats as individuals with internal states, and to develop more positive attitudes and a sense of commitment and responsibility for them [137]. The literature on human–animal relationship indicates that, among the many factors involved in determining attitudes towards other species, the belief in animal sentience or "mind" is a strong predictor of human attitudes toward different types of animal use [144–146].

Unfortunately, there is a lack of information about communication and interactions between humans and the "stem species" of domesticated goats—the wild goat *Capra aegagrus*. Therefore, it is not possible to compare the response behaviour of wild goats to humans with the behaviour of domestic goats. Further studies on behavioural interactions between humans and the still-existing wild ancestor of goats would be extremely useful to better understand and interpret the behavioural responses of domestic goats and to better understand the mechanisms and evolution of the human–goat relationship.

From the management point of view, there are several points where humans can have an active role to improve HAR. First of all, we have found five studies [17,54,80,84,85] highlighting that hand rearing of goat kids can have a positive effect on several aspects of the human–goat relationship. This apparently suggests to hand rear goat kids. However, there is a growing consumers' hostility to the early separation of young animals from their mothers in the dairy industry. In cattle farming, early interactions with calves kept in cow–calf contact systems (e.g., assistance with suckling) is recommended to reduce future fear of humans [147]. The avoidance distance in cow-reared calves is shown to decrease with no differences compared to artificially reared calves in later ages (from calves to primiparous cows). Although there is a lack of specific studies in goats on this topic, it seems reasonable to think that raising animals with their mothers is not an obstacle to the establishment of a good HAR, but it does require specific knowledge from the farmer and, above all, humans have to keep in mind the importance of early contacts with kids, especially during their sensitive stages. In fact, frequent handling and frequent contacts between humans and goats have been found to improve the human–goat relationship [22]. Of course, it is also important to consider not only the frequency, but also the nature of the interactions. Gentling treatments, such as friendly talking, gentle touching, stroking and frequent hand feeding [19,48], may have a positive effect on HAR, whereas negative interactions, such as talking harshly, hitting and kicking the goats [87] have a negative impact. Furthermore, maintaining a low goat/stockperson ratio on farm, which implies more frequent opportunities for farmers to interact with their goats, also allows for the establishment of a more positive relationship [88].

Furthermore, as goats have well marked individual traits and temperaments, humans should learn to pay attention to individual animals, and genetic selection toward genotypes with a calm temperament, high docility and low timidity may also help to improve HAR.

A further useful way to improve the human–goat relationship would be to focus on the human side, understanding which farmers' characteristics and behaviour allow to better combine good productivity, good practice and appropriate welfare standards. Farmers' competence and technical skills, job motivations and satisfaction and attitudes combined with personality traits have been identified as job-related prerequisites for ensuring appropriate farm animal welfare standards [98,101]. These characteristics have been scarcely investigated in goat farmers so far (see [92,93,113]) and need to be further explored in future research. Sociodemographic variables (e.g., gender, cultural level) and personal characteristics (personality, empathy, and attitudes) have been shown to affect humans' behaviour towards animals [93,95,101]. Gender differences have been well documented in the literature, with women showing higher levels of concern about animal suffering, holding more positive attitudes towards animals and being more engaged in animal protection and less prone to animal exploitation (see [103,148] for reviews). Since this has been confirmed also in goat farms [92], a greater involvement of women in goat farming should be welcome and fostered. Moreover, training programs aimed at promoting farmers' development of positive feelings and empathic concern towards goats could be developed. Goat stockpersons' personality (i.e., their pattern of thoughts, feelings and behaviours) has not been investigated; however, personality traits affect human behavioural responses towards both people and animals and relate to both animal welfare and productivity (e.g., [96]). Goat farmers' attitudes deserve investigation, since attitudes are key determinants of human behaviour towards people and animals, and their influence on interactions with farm animals, productivity and management has been shown for different farmed species (e.g., pigs: [149]; cattle: [96,150,151]). It is worth noting, however, that stockpersons' attitudes toward animals are not just limited to the direct interactions with them, such as handling, but also affect attention to details, readiness to solve problems, decisions in management and housing decisions; thus, understanding the role of attitudes in goat farming would be particularly useful. Given that attitudes are learnt and may change over time because of new information, new experiences and newly acquired knowledge [152], appropriate training based on specific experiences and knowledge acquisition could have a positive effect in changing farmers' attitudes, reducing negative behaviours and poor relationships. According to [15], to ensure that training programmes are well targeted for farmers, they should be adapted to situations, needs and level of knowledge that humans have already acquired.

In practice, while there is a fair number of validated measures for assessing the human–goat relationship, many of these are recorded by unfamiliar assessors, and they are not designed for a feasible self-assessment from farmers or owners. However, knowing the meaning of some of the goats' reactions towards humans, e.g., avoidance and flight reaction or voluntary approach to a familiar person, can help to identify possible issues. In addition, the behaviour of goats in the presence of unfamiliar people (e.g., visitors, children from schools) can also be observed to see how goats perceive the human presence and if the animals need to be more accustomed to people. A good human–goat relationship implies a number of work-related aspects including competence and technical skills, which are important also in implementing farmers' job satisfaction. Job satisfaction is associated with more positive attitudes and emotions, and with greater farmers' self-efficacy and wellbeing [153–155]. Farmers' job satisfaction and wellbeing can be viewed as prerequisites for optimal stockmanship and should be investigated and implemented to improve goat welfare. As claimed by [155], animal welfare is a multi-faceted issue with scientific, ethical, economic and political dimensions, and it requires a multidisciplinary approach to broaden the understanding of the human–goat relationships aimed at improving the lives of both humans and animals.

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2. Indicators of emotions in small ruminants

In humans, the conscious experience of emotions triggers changes at the neural, behavioural, physiological and cognitive levels that can be objectively measured through linguistic self-report. In animals, the inability to access the conscious perception of emotions requires the use of indirect indicators that measure the same changes that emotions induce in humans (Mendl et al., 2010). However, this is a process of inference based on the assumption that a measure associated with an affective state in humans is also associated with the same affective state in non-human animals. This perspective is undoubtedly anthropocentric and could lead us into error, especially with regard to animal species that are phylogenetically more distant from humans. Another limitation of this perspective is that we may miss indicators of emotions for which we have no sensory experience (Mendl et al., 2022).

2.1 Vocalizations

With the exception of a few animals, such as "talking" parrots and some great apes, animals are unable to communicate their affective states using language. However, vocalisations represent a natural emotional behaviour of animals and can be used as a proxy for the expression of affective states (Panksepp, 2011).

2.1.1 Source-filter theory of voice production

The source-filter theory of vocal production (Fant, 1960; Titze, 1994) was formulated in relation to the human language and provides a framework that links speech production, acoustic structure of the sound, and functional discrimination/perception (Taylor and Reby, 2010). According to this theory, human speech is produced as part of a two-step process involving breathing, phonation, resonance and articulation. The lungs generate an air flow which passes through the larynx (source), causing the vocal folds to vibrate. The vibration of the vocal folds produces the sound (the waveform of which is the source signal or glottal wave). The sound is then filtered at the level of the supralaryngeal vocal tract (i.e., the filter, consisting of the pharynx and the oral and nasal cavities) and then emitted into the environment. The involvement of breathing, phonation, resonance and articulation in speech production and their influence on the acoustic characteristics of sounds are schematised in Table 2 (Briefer, 2012a; Taylor and Reby, 2010).

Table 2. Processes and anatomical parts involved in vocal production according to the source-filter theory (Briefer, 2012a)

	Process	**Anatomical parts involved**	**Vocal parameter associated**
Source	respiration	lungs	Duration, rate, amplitude, f_o
	phonation	larynx, sub-laryngeal and laryngeal structures	f_o contour
Filter	resonance	all the air cavities between the larynx and the mouth/nostril opening (vocal tract)	Frequency spectrum characteristics and formant contour
	articulation		

As shown in table 2, the source of voice production, the larynx, is responsible for generating the fundamental frequency of the sound; subsequently, the vocal tract shapes the glottal wave by amplifying some frequencies and attenuating others (Briefer, 2012a). The formants, i.e., the peaks of the acoustic spectrum, originate from this filtering mechanism. The source-related vocal parameters depend on the anatomy and physiology of the larynx (vocal fold length, thickness, mass, tension and internal structure, i.e., collagen and elastin fibre density and orientation), whereas the filtering parameters depend on the anatomy and physiology of the vocal tract (its shape, length and tension; Briefer, 2012a).

The generalisation of the source-filter theory of vocal production to non-human animals, particularly mammals, is based on the similarity of their vocal production apparatus (Titze, 1994). This theory allows the identification of certain static information about the emitter (Coutand et al., 2024), such as its individuality, size, weight and sex (Taylor and Reby, 2010), but also dynamic information, including its affective state (Coutand et al., 2024). Since the source-filter theory of speech production allows us to define how acoustic parameters are generated, i.e., depending not only on the anatomical, but also on physiological characteristics of the caller (e.g., respiration, action or tension of the respiratory muscles), it allows us to assess the motivations behind their change, such as arousal and emotional valence (Briefer et al., 2020).

2.1.2 Influence of emotions on vocalisations in small ruminants

The presence of acoustic markers of emotions in the human voice is known as “affective prosody”. The vocal expression of emotions in animals was first suggested by Darwin in 1872. Later scientific studies into the process of encoding the information contained in vocalisations confirmed that there are certain vocal parameters that can be considered reliable indicators of emotion

(Briefer, 2012a). Emotions affect vocalisations because they affect all four systems involved in vocal production (changes in respiration and salivation, and in the tension and action of the muscles involved in the processes of phonation, resonance and articulation). These changes are generated by emotionally induced changes in the somatic and autonomic nervous systems (Scherer, 2003). Vocalisations can be considered as good indicators of emotions because they are “honest” signals, as animals are less able than humans to control vocal production. Vocalisations can be collected from a distance and are not affected by the presence of obstacles in the environment. Furthermore, they have the potential to provide real-time information about animals’ affective state on farm thanks to Precision Livestock Farming (PLF) technologies (Briefer, 2020). Emotional arousal underlies changes in acoustic parameters associated with respiration and phonation, such as the fundamental frequency, amplitude and temporal parameters of the sound (i.e., duration and rate; Scherer, 1986), while the emotional valence influences the distribution of energy in the spectrum, i.e. its formants (Scherer, 2003). In small ruminants, there is little research on the influence of emotional arousal and valence on the vocal parameters of bleats (i.e. vocalisations produced by these animals). Table 3 summarises the main findings on this topic that were obtained from literature searches using the keywords goat* OR "*Capra hircus*" AND emotion* OR "affective state*” AND vocal* OR bleat* OR acoust* OR sound* OR "vocal parameter" in the Web of Science and Scopus databases.

Table 3. Changes of the vocal parameters in goats and sheep bleats depending on their emotional valence and arousal

Species	Valence (from negative to positive)	Arousal (from low to high)
Goat	More stable f_o (<f_o range, < f_o extent; Briefer et al., 2015a) > call rate (number of vocalizations in the time unit; Baciadonna et al., 2020)*	> call rate (number of vocalizations in the time unit; Briefer; 2015a) > f_o (Briefer et al., 2015a) > f_o contour parameters (f_o*mean* and f_o*end;* Briefer et al., 2015a) < First formant (*F1*) contour < frequency range (Siebert et al., 2011) > energy distribution (i.e., energy quartiles = Q25, Q50 and Q75; Briefer et al., 2015a) < entropy (Siebert et al., 2011)
Sheep	-	Production of high frequency (open-mouth vocalizations; Dwyer, 2008; Engeldal et al., 2013; Papadaki et al., 2021) > f_o contour parameters (Sèbe et al., 2012)

	> fundamental frequency related parameters (> $f_o$min, > $f_o$mean and > Q50%; Papadaki et al., 2021) > fundamental frequency disturbance (> Root mean square of the amplitude (RMS), > jitter and shimmer values; Papadaki et al., 2021) < energy distribution (i.e., energy quartiles = Q25, Q50 and Q75 (Sèbe et al., 2012; Engeldal et al., 2013)

Both goats and sheep produce closed-mouth bleats, also called low-frequency bleats, and open-mouth bleats, also called high-frequency bleats. Goats also produce two other types of vocalisations, represented by gobbles, which are part of sexual behaviour, and alarm snorts. In both species, open-mouthed bleats do not allow to infer the valence of the emotional state experienced by the animals, as they are produced in both positive and negative valence contexts (Lenhardt, 1977; Dwyer, 2008). For example, high-frequency bleats are produced by ewes both for conspecific recognition and in high-arousal contexts (Papadaki et al., 2021; Dwyer, 2008). Low frequency bleats ('rumbles') are produced by ewes in the presence of the lamb for the purpose of recognition and/or reinforcement of the mother-lamb bond (Dwyer, 2008). Although these vocalisations are emitted in positive-valence contexts, they should be considered specific to this context and this may, in my personal opinion, be the reason why they do not appear as an indicator of valence in the sheep species. It should also be noted that no acoustic parameters indicating emotional valence have been identified in this species yet (Table 3). The literature search showed similarities in the call rate changes of goat vocalisations emitted in positive contexts (Baciadonna et al., 2020) and in contexts characterised by high arousal, regardless of emotional valence (Briefer et al., 2015a). This is an interesting aspect that requires further research. The opening of the goat's mouth would be the cause of the reduction of the F1 contour, as suggested by Briefer et al. (2015a), who point out that energy quartiles (Q25-Q75%) are easier to measure than fundamental frequency or formants, and therefore suggest their use as indicators of emotional arousal for the goat species (Briefer et al., 2015a). Table 3 shows the variation in acoustic parameters of bleats in sheep and goats for which there is agreement in the scientific literature.

2.1.3 Perception and contagion of vocal expressions of emotion

Emotional contagion occurs when the transfer of emotions from one subject to one or more subjects results in a correspondence, i.e., synchronisation, of their emotional state (Briefer, 2018).

In social and gregarious species, such as goats and sheep, the simultaneous expression of the same affective state has an adaptive function (Briefer et al., 2012b), as the composition and size of social groups are affected by changes over time, and can also be regarded as the first stage of empathy (Briefer, 2018). Emotional contagion (which occurs not only through vocalisations, but also, for example, through facial expressions and olfactory signals) has important social implications, as it promotes communication, coordination and cooperation among animals (Spinka, 2012). Vocal contagion of emotions has both direct or unconditioned effects, represented by the Acoustic Startled Reflex and capturing the attention of a conspecific to influence its arousal level, and indirect or learned effects, represented by affective learning and learned affect, which occur through conditioning (see Briefer, 2018 for more details).

For emotional contagion of vocal expression to occur, an individual must be able to perceive and decode the emotional content of another individual's vocalisations (Briefer, 2018). This perceptual ability has been found in goats, which are reported to be able to discriminate between vocalisations of different valence from conspecifics. Baciadonna et al. (2019b) used a specific type of playback paradigm, a habituation-recovery paradigm, through which they showed that goats are not only able to discriminate between positive and negative bleats (greater tendency to look in the direction of the sound in response to a change in the valence of the sound), but are also affected by them, as evidenced by the increase in heart rate variability as a result of listening to positive bleats. In another study, Baciadonna et al. (2019a) showed an involvement of the left cerebral hemisphere of goats (i.e., lateralised attention to acoustic stimuli) in decoding conspecific vocalisations. The researchers concluded that the left hemisphere is therefore involved in the processing of vocalisations that are perceived as positive, or at least not alarming (Baciadonna et al., 2019a). However, this study did not test the correspondence between the emotional state of the caller and that of the receiver of the vocalisations; in this case, the researchers would have expected a different involvement of the cerebral hemispheres depending on the valence and emotional arousal of the calls (Baciadonna et al., 2019a). Although the studies mentioned above refer to emotional valence discrimination, there is more evidence in the scientific literature on vocal contagion of arousal (Briefer, 2018). The literature search conducted by using the keywords goat* OR "*Capra hircus*" AND emotion* OR “affective state” AND vocal contagion in Web of Science and Sopus databases did not reveal any other studies investigating perception and emotional contagion mediated by vocalisations in goats, and there are no studies in sheep (the keyword sheep OR "*Ovis aries*" OR ovine OR lamb* was used for this species). The project ‘Vocal contagion of emotion in sheep’, carried out by the University of Copenhagen in collaboration with INRAE, aims to shed light on how emotional states encoded in acoustic information can propagate at flock level and how they influence the movements of the group (https://www.behavioural-ecology-group.com/phd-postdoc-projects/). Part of the results of this project are reported in the present thesis (5.3 Acoustic markers of negative arousal in lambs (*Ovis aries*): evidence from behavioural and eye thermal profiles).

2.1.4 Cross-species expression and perception of emotions through vocalizations

The universality of vocal expression of emotion in animals, particularly in mammals, has been studied as a function of the motivational state of the emitter ("motivation-structural rules") or of the two dimensions that characterise emotions, namely valence and arousal level ("emotional-dimension rules"; Briefer, 2020). To date, scientific studies have focused on identifying vocal correlates of emotional arousal rather than valence, and mostly in negative contexts (Briefer, 2020). However, as mentioned in section 1.6.1, the new concept of "positive" animal welfare requires that animals can also experience positive affect, and therefore the identification of vocal parameters associated with valence is an important step. It should be noted, however, that it is more difficult to detect calls emitted in positive contexts than in negative ones, and that valence validation would also require the collection of vocalisations with similar levels of arousal (Briefer, 2020). In 2012, Briefer published a review on the vocal expression of emotions in mammals: of the 58 studies considered, only eight examined emotional valence, and almost all of them involved companion animals or wildlife. Since 2012, the scientific community's interest in the vocal correlates of emotional valence has increased, and several studies have also been published on different farm animal species, such as goats (Briefer et al., 2015a), domestic horses (Briefer et al., 2015b), pigs (Briefer et al., 2019; Briefer et al., 2022) and cattle (Meen et al., 2015). A review by Briefer (2020) compared how vocal parameters change with varying arousal and emotional valence in different animal species. If we want to generalise, it can be argued that as the arousal of a situation increases, calls become louder and they are mitted at a faster rate. In addition, they are characterised by higher frequencies (both fundamental and other frequencies of the spectrum) and have a more variable fundamental frequency (f_o). In terms of valence, the calls emitted in positive situations are shorter and probably have a lower and less variable fundamental frequency, although duration is the vocal parameter that has shown greater consistency in the reviewed studies (Briefer, 2020). In goats, as well as in Przewalski's horses, the fundamental frequency range (f_o range) has been identified as an indicator of valence; however, the call duration was not found to be an indicator of valence in goats (Greenall et al., 2022). As mentioned in 2.1.2, there is currently no evidence for vocal indicators of valence in sheep.

Regarding the intra- and heterospecific recognition of emotions through vocal production, scientific studies showed that goats are not only able to recognise the emotional valence of calls emitted by conspecifics (Baciadonna et al., 2019b), but also that of the human voice (Mason et al., 2024). The same ability has been observed in domestic horses, Przewalski's horses and domestic pigs, either as a result of phylogenetic processes that have allowed the conservation of vocal parameters of emotional valence during evolution (Equidae), or as a result of the process of domestication, which has improved the capacity of some species to interpret human vocal cues (domestic pigs; Maigrot et al., 2022).

Greenall et al. (2022) investigated the ability of humans to distinguish between high vs low arousal and positive vs negative valence in vocalizations emitted by several species. This is the only study conducted on the ability of humans to recognise the emotional expression of goat vocalisations and shows that humans are able to recognise both the emotional valence and the arousal of bleats (recognition rate significantly above chance level), albeit at rather low rates (56 and 57% respectively; Greenall et al., 2022). In this study, individual factors such as familiarity with the species, age between 20 and 39 years and high empathy were found to influence the correct assignment of goat vocalisations to situations of positive vs negative valence and of high vs low arousal. This was consistent with the findings on the other domestic (pigs, horses, cattle) and wild (Przewalski's horses, wild boars) species, and of humans, investigated in the same study. Vocalizations emitted by domestic species were generally more recognised than those from wild species. Interestingly, certain acoustic parameters also improved the correct recognition of the arousal and valence of the goats' bleats. In particular, the greater difference in spectral centroid and the smaller difference in terms of duration between the two sounds the participants had to evaluate positively affected the recognition of the arousal level. Valence was also assigned more correctly when the differences between the two sounds were smaller in terms of duration, fundamental frequency (f_o) and amplitude modulation (*AM*). In addition, the acoustic characteristics of the bleats influenced people's perception of valence and emotional arousal: bleats perceived as at a higher arousal were longer in duration, had a higher spectral centroid, a higher f_o and a lower *AM*. In contrast, bleats perceived as more positive were characterised by a higher spectral centroid and lower amplitude modulation (*AM*). The present thesis includes the second study carried out so far on the human perception and interpretation of emotions conveyed by goat vocalisations (see section 5.2. "Human perception of the vocal expression of emotions in goats"; Celozzi et al., 2024; in preparation), which contributes to the study of interspecies communication.

2.2 Facial expressions

Darwin investigated animal emotions through vocalisations as well as tears and postures, but his main contribution to our understanding in this field was the study of facial expressions (Darwin, 1872; Ekman, 2009). Many of the photographs of human emotional facial expressions in “Expression of Emotions in Man and Animals” were taken by the French neurologist Duchenne De Boulogne and included in Darwin's work with his permission (Ekman, 2009).

More than a hundred years later, Duchenne's photographs provided the basis for the development of a tool based on the anatomy of the human face to describe and measure facial movements: the Human Facial Action Coding System (FACS; Ekman & Friesen 1978). This tool allows the identification of minimal facial movement units, also known as Action Units (AUs), each based on a specific muscle movement, or on a combination of movements. This methodology is standardised and objective and has been adapted for eight species: chimpanzees, macaques, gibbons, orangutans, dogs, cats, horses and marmosets (https://animalfacs.com/). However, it is questionable whether FACS can be used to quantify animal emotions (Waller, 2023).

The concept of action units has been adopted by another method in the study of facial expressions, namely grimace scales, to indicate areas of the face that change in response to certain emotional states (McLennan et al., 2019), of which pain has received the most attention. Pain-identifying grimace scales have been developed for several species, including rodents, rabbits, ferrets, cats, pigs and horses (Langford et al., 2010, Sotocinal et al., 2011, Keating et al., 2012, Reijgwart et al., 2017, Holden et al., 2014, Di Giminiani et al., 2016, Dalla Costa et al., 2014, van Loon and VanDierendonck, 2015).

In small ruminants, pain grimace scales have been developed in adult sheep (Sheep Grimace Scale (SGS); Häger et al., 2017; Sheep pain facial expression scale (SPFES); McLennan et al., 2016), lambs (Lamb Grimace Scale (LGS); Guesgen et al., 2016), and goats (Goat Grimace Scale (GGS); Weeder et al., 2023; Table 4). Most of these scales were developed by inducing a painful stimulus, except for one study based on changes in AUs in sheep naturally affected by footrot and mastitis (McLennan et al., 2016). Table 4 summarises the emotion-generating contexts and changes in action units considered in the facial scales developed for sheep and goats. Of all the facial areas shown to be indicative of pain, the eyes and ears were always assessed in all scales, as their degree of aperture and posture have also been used in other scientific studies as facial indicators of emotion in these species.

Table 4. Facial scales developed for the evaluation of signs and its intensity of pain in sheep and goats

Species	Method	Emotion	Emotion-generating context	Ears	Nose	Eyes/ Orbital tightening	Head position	Flehming	Abnormal lip and jaw profile	Check (masseter muscle tightening)
Sheep	**Sheep Grimace Scale (SGS)** Häger et al. (2017)	pain/ distress	unilateral tibia osteotomy	moderate pain: flattened severe: hanging	-	moderate pain: half-closed severe pain: completely closed	moderate pain: slanted head severe: hanging	severe pain: presence of flehmen	-	-
	Sheep pain facial expression scale (SPFES; McLennan et al., 2016)	pain	naturally-occurring diseases (footroot and mastitis)	fully rotated ventrally and caudally; inner pinna less visible	"V" shape between nostrils apertures (due to shortening and narrowing of the philtrum)	closure of the palpebral fissure by the eyelids; narrowing of the eye aperture	-	-	concave jaw profile; straight or rotated ventrally lip line	more convex shaping of the the cheek
	Facial Action Unit Detection (Lu et al., 2017)	pain	none (a dataset of sheep faces labelled on the basis of SPFES AU's taxonomy)	moderate pain: rotated, pinna less visible severe pain: flipped, pinna not visible	moderate pain: shallow; V shaped nose severe pain: extended, V shaped nose	partially closed	-	-	-	-
	Lamb Grimace Scale (LGS) (Guesgen et al., 2016)	pain	tail docking	tense ears, pointed backwards or downwards	tightened nose with a decrease in nostril size (horizontal line	"squeezing"/closing of the eye	-	-	flattened and tightened (no "upwards curl"	Cheeks less rounded

					or V shape nose appearance)				at the edge of the lips)	
Goats	**Goat Grimace Scale (GGS)** (Weeder et al., 2023)	pain	lameness (induced)	asymmetrical or pointed backwards	more or less narrowed nostrils; partially curved or “V” shape nostrils	Eye more than half closed with tension around the orbit	-	-	-	Protruding-looking cheek

Horizontal ear posture (or "passive, non-erect ears") has been observed in sheep in positive emotional contexts (i.e. grooming, eating a palatable food; Reefman et al., 2009a; Reefman et al., 2009b). In contrast, more frequent changes in ear postures were associated with the experience of negative emotions, such as separation from group members (Reefman et al., 2009a; Reefman et al., 2009b). Boissy et al. (2011) associated certain ear postures in sheep with discrete emotions, suggesting that ears backwards would indicate fear, ears forward would be associated with anger, while asymmetrical ear postures would express surprise. However, in these studies, the emotion-eliciting contexts may have differed not only in valence, but also in arousal level. In a more recent study, using positive and negative contexts with similar levels of arousal, sheep in a negative mood moved their ears more and had more ears forward and less ears backward after being exposed to a negative stimulus (Vogeli et al., 2014).

Briefer et al. (2015a) identified goats' ear postures as potential indicators of emotional valence and arousal. In situations characterised by high levels of arousal, goats are more likely to have ears pointing forward and less likely to have horizontal ears pointing on the sidely, while in positive situations they are less likely to have ears pointing backwards. Although Bellegarde et al. (2017) observed more forward-pointing ears in goats when viewing conspecific faces photographed in negative rather than positive situations, the authors suggest that this behaviour probably indicates a high level of arousal due to a state of vigilance and attention, rather than to the negative valence of the context.

Studies in sheep show a reduction in eye aperture in response to gentle stroking, a context presumably characterised by positive valence and low arousal (Reefman et al., 2009b). However, the Grimace Scales show how pain, an emotion characterised by negative valence, leads to partial eye closure as a result of orbital stiffening. Furthermore, eye aperture seems to increase with arousal, regardless of the context valence (Reefman et al., 2009b; Reefman et al., 2009c).

2.3 Posture and body language

In goats, an increase in head and body movements has been associated with an increase in the level of arousal, regardless of the emotional valence of the context (Briefer et al., 2015a). In addition to higher rates of locomotion, Siebert et al. (2011) observed higher rates of rearing and jumping in goats in partial isolation compared to full isolation. Partial isolation in this study is likely to induce a higher level of arousal than complete isolation, as the constant sensory feedback from conspecifics housed in adjacent pens is supposed to underlie the increased motivation to re-establish social contact with them. Gygax et al. (2013) did not observe an increase in locomotor activity in goats exposed to a high-arousal positive stimulus (uncovered feed bowl), because its high attractiveness elicited more stimulus-directed behaviours. In contrast with Siebert's findings

(Siebert et al., 2011), according to Gygax et al. (2013) rearing did not discriminate the valence of the stimuli to which the goats were exposed. In sheep, increased locomotor activity is also indicative of an active response of the animals to a high-arousal condition such as social isolation (Boissy et al., 2005), although to my knowledge there are no studies that clearly describe the variation of bodily activation with arousal in contexts of similar valence in this species.

Tail position was found to be a proxy for emotional valence in goats, which spent more time with the tail raised in positive than in negative situations (Briefer et al., 2015a). In contrast, Reefman et al. (2009a) recorded a high frequency of raised tail in sheep during the separation from group members, whereas this posture was not observed during rumination (intermediate-valence context) or fresh hay feeding (positive-valence context).

2.4 Physiological indicators (biomarkers)

Physiological indicators of affective states can be studied within four physiological subsystems: the neuroendocrine, autonomic, immune and metabolic systems. The field of research investigating the involvement of the neurological system in the domain of affective states is called affective neuroscience (Panksepp, 1998) and is concerned with the study of the neural mechanisms of emotion and of the neuropeptides involved in emotional processes. Psychophysiology, on the other hand, is concerned with more peripheral markers of emotion (Kremer, 2023).

In goats, some physiological parameters related to the autonomic nervous system that can be used as markers of affective states have been identified by Briefer et al. (2015a), who observed that heart rate variability (RMSSD; root mean square of successive interbeat interval differences) decreases and respiratory rate increases with arousal. As these two physiological parameters are not correlated, they can be considered as independent indicators of arousal in goats. RMSSD and heart rate (the latter was not found to be an indicator of arousal) are involved in the activation of the Sympathetic-Adrenal-Medullary axis in response to stress (Briefer et al., 2015a).

In sheep, there are studies (see below) that show the variation of certain physiological parameters with valence, but without controlling for the level of arousal (i.e., the contexts used were different for both emotional valence and arousal). On the other hand, other studies consider only one emotion-generating context, making it impossible to draw conclusions about the potential use of physiological indicators as markers of valence and arousal in this species.

Heart rate variability was found to be higher in a positive context (grooming) and decreased linearly in a negative context in ewes (separation from group members; Reefman et al., 2009b), whereas increased heart rate was observed in negative contexts, represented by the feeding of an unpleasant feed (wood pellets) and regrouping (Reefman et al., 2009a; Papakitsos et al., 2023).

Consistently with these findings, lambs stroked by humans (i.e., positive context) showed slower heart rates and higher heart rate variability (Coulon et al., 2015).
The inverse measure of heart rate, mean interheartbeat intervals, was found to be lower in negative than in positive contexts (separation from conspecifics vs grooming; Reefman et al., 2009b). In addition, respiration rate and body surface humidity variability were found to be higher in response to the administration of a low-palatable than of a high-palatable food (Reefman et al., 2009a).

2.5 Cognitive bias

Affective states affect animals' cognitive processes (Baciadonna et al, 2016) and can be assessed through cognitive biases such as attention (or attentional) bias, memory bias and judgment bias (Paul et al., 2005). Attention bias compares the level of attention paid by the subject to threat-associated stimuli, which is higher than that paid to non-emotional stimuli (Eysenck and Keane, 2020). Memory biases, on the other hand, are based on the greater ease of remembering events associated with positive and negative emotions than emotionally "neutral" events, and involve the processes of memory storage, consolidation and retrieval (Roelofs al., 2016). In judgement bias, an animal's judgement of an ambiguous stimulus is influenced by its emotional state, with the experience of negative emotions or a negative mood (i.e., depressive or anxious states) leading to a "pessimistic" judgement of the stimulus (Richards and French, 1992).
A bibliographic search using the keywords goat* OR "*Capra hircus*"/sheep OR "*Ovis aries*" OR ovine OR lamb* AND emotion* OR "affective state*" AND "memory bias"/"attention bias" OR "attentional bias" was carried out in the Web of Science and Scopus databases; it was found that memory bias has never been used to assess emotional states in small ruminants, whereas attention bias has only been used in sheep to measure anxiety and depression. Monk et al. (2018) observed that sheep pharmacologically-induced into a depressive or anxious state showed differential modulation of attention to a threatening stimulus (dog). Depressed sheep were more attentive to the threatening stimulus, and both depressed and anxious sheep showed increased vigilance behaviour. In another study, Monk et al. (2020) observed that these cognitive biases did not allow to discriminate between positive and negative affective states in sheep, and therefore could not be used as indicators of emotional valence in these species. In another study, exposure of sheep to a chronic stress (i.e., disruption of rest and individual housing) resulted in reduced attention to a threatening stimulus and increased reward expectation (Verbeek et al., 2019).
As to judgment bias in sheep, Doyle et al. (2010) found that restraint and isolation (RIS) were associated with positive judgement in sheep, contrary to expectation. However, the authors suggest that release from restraint and isolation may have produced a positive emotional state in sheep,

similar to that observed in goats after the end of a lifetime of poor care (Briefer et al., 2013). Another hypothesis to explain these unexpected results is that the negative effects of RIS were not permanent when sheep were tested. In a study conducted by Sanger et al. (2011), opposite results were found in two cohorts of animals (each with an experimental group - sheared animals and a control group - non-sheared animals), as only in the first cohort the release from shearing stress induced positive emotions that were associated with an optimistic judgement. According to the Authors, this may be explained by the fact that the control group of the second cohort had witnessed the shearing of the experimental group of the first cohort the day before, and this may have induced a state of anxiety that justified a positive judgement when subjected to the judgement bias test. In Destrez et al. (2012), the exposition to chronic stress for nine-weeks (presence of a dog, odour of a killed conspecific, and human handling procedure) was associated with a pessimistic bias in sheep to both ambiguous and negative stimuli. In Verbeek et al. (2014), feeding restriction induced an optimistic bias in the sheep, possibly due to the limited duration of the treatment (7 days), which would therefore not induce a negative affective state.

In conclusion, from the above-mentioned studies it seems that the results of experiments using of attention bias and judgement bias as indicators of sheep emotions are mostly inconsistent, although they may possibly be explained by the different experimental conditions of the studies (i.e., type of task, type of stimuli used), and certainly deserve further studies in more standardised conditions.

In goats, Briefer and McElligot (2013) observed an optimistic bias in the evaluation of an ambiguous stimulus in subjects who had experienced prolonged neglect and/or abuse prior to arriving at a sanctuary. Although these results contradict the *a priori* hypothesis, the stress relief experienced by the goats in their new environment would underlie their increased expectation of the occurrence of positive events, although sex differences were observed in this study. In contrast, the excellent living conditions experienced by the goats prior to performing a judgement bias test in a study by Baciadonnaet al., (2016) could explain the lack of optimistic response in the goats after experiencing a short-term positive affective state (human grooming). The stimulus in question would be perceived as positive by the goats, but this emotional dimension was not detected during the test.

Images of conspecific faces in positive, negative and ambiguous emotional situations (faces morphed to have an intermediate facial expression between positive and negative) were used by Bellegarde et al. (2017) in a judgement bias paradigm. Subjects were able to discriminate between positive and negative situations, but they did not show an intermediate behavioural response when exposed to the ambiguous faces of conspecifics, leading the authors to conclude that further research is needed on this topic.

2.6 Qualitative Behaviour Assessment

Qualitative Behaviour Assessment (QBA) is an integrative methodology for assessing the way in which animals express their behaviour. This method uses and an integrative approach and looks at the "whole animal", integrating animal behaviour and its interaction with the surrounding environment. QBA assesses animal behaviour using a qualitative terminology, through descriptors such as "calm", "anxious", "timid". These terms have expressive and emotional connotations that provide insight into how an animal experiences a situation and are therefore relevant to the assessment of welfare status and emotions. These descriptors should not be considered as causal factors of a physical or mental state, but should take into account feelings, genetic predispositions, cognitions and any other single factor in the context of an "integrated state" (Wemelsfelder et al., 2001).

QBA is one of the indicators included in the protocols for assessing the welfare of adult sheep kept indoors or outdoors (AWIN, 2015a) and adult dairy goats kept in intensive or semi-intensive production systems (AWIN, 2015b), to assess the welfare criterion of positive emotional state within the welfare principle of appropriate behaviour. In the AWIN protocols, the assessment of the qualitative expression of behaviour refers to a group of animals rather than to an individual, and is performed by scoring qualitative descriptors (13 for goats and 21 for sheep) using a visual analogue scale (VAS).

QBA has been used in several scientific studies to assess welfare in on-farm settings for both goats (Battini et al., 2018) and sheep (Phythian et al., 2016), and to assess stress during transport in sheep (Collins et al., 2018). The indicator has also been used for young animals (see e.g. Napolitano et al., 2018 for goat kids and Bodas et al., 2021 for lambs), a category of animals not included in the AWIN protocols. In addition to its use to assess global welfare status, QBA has also been used to assess the behavioural expression of animals following specific events, such as changes in housing conditions (Napolitano et al., 2018), to assess the adaptation of feral goats to an intensive housing system (Miller et al., 2018).

QBA is a reliable and repeatable method under controlled experimental conditions (Wemelsfelder and Lawrence, 2001), so assessing the intra and inter-observer reliability of this method on the farm is an important step for its successful application, as is cross-validation with "conventional" indicators of animal welfare (Wemelsfelder and Lawrence, 2001). Studies in this sense have been carried out both in goats (Grosso et al., 2016) and in sheep (Phythian et al., 2013).

2.7 Monitoring emotions in small ruminants using Precision Livestock Farming

Precision Livestock Farming (PLF) is a multidisciplinary field combining engineering, computer science and statistics applied to agricultural systems. These systems include not only crops, water and soil, but also animals, that are the subject of precision livestock monitoring. The field of engineering includes the use of sensors to collect animal data. These can be on-animal sensors, such as wearable sensors, or off-animal sensors, such as robots, cameras or Internet of Things (IoT) (Trotter et al., 2022; Dorea, 2024). Computer science includes the development of algorithms, the application of machine learning techniques, the creation and storing of databases and CPUs/GPUs (i.e., Central Processing Units and Graphics Processing Units, respectively). Finally, statistics applied to PLF includes various techniques such as causal inference, experimental design, ANOVA and regression (Dorea, 2024). The engineering and computer science components of PLF are part of Artificial Intelligence (AI), i.e., "a machine-based system that, for explicit or implicit objectives, infers, from the input it receives, how to generate outputs such as predictions, content, recommendations, or decisions that can influence physical or virtual environments. Different AI systems vary in their levels of autonomy and adaptiveness after deployment" (OCSE, 2024).

Precision livestock farming aims to provide real-time monitoring of animals kept under farming conditions at two levels: individual (animal level) or group (farm or pen level). In addition, precision livestock aims to optimise management to improve product quality and production efficiency, animal welfare and environmental sustainability. Finally, it also plays an important role in genetic improvement through the identification of new traits, better evaluation and the ability to monitor the interaction between the animal's genotype and the environment (Dorea, 2024).

Of the one hundred and ninety scientific articles published between 1999 and 2017 on sensing technology to collect information at the animal-level, it was possible to identify a bias in its use in relation to bovine species, in particular dairy cattle (92.6% of articles). Within this livestock sector, oestrus, lameness and mastitis were the main applications (65% of articles). Since the advent of technology in the livestock sector, wearable sensors, particularly accelerometers, have been the most commonly used sensors, and behaviour the most commonly monitored phenotype (Dorea, 2024).

The development and diffusion of precision livestock technologies in small ruminant husbandry has been scarcer and more limited than in more economically important livestock sectors, such as cattle, pigs and poultry (Caja et al., 2020). The main barriers are the low payback capacity of the technologies in small realities such as sheep and goat farms, and the lack of equipment specifically designed for sheep and goats. On the other hand, the use of technologies, such as those allowing remote monitoring of animals, would be particularly useful in the sheep and goat sector, as these animals are often reared in extensive/semi-extensive systems (Caja et al., 2020). In fact, Spigarelli et al. (2020) highlight that the use of PLF represents a very promising opportunity to collect welfare measures in these kind of systems, where direct and close access to the animals is sometimes difficult, and where the collection of individual records may be very time-consuming and may require operations such as herding and restraining, that are potentially stressful for the animals.

Caja et al. (2020) provide an overview of the wearable and non-wearable sensors used in the small ruminant sector and their main areas of application; among the latter, none relate to monitoring the emotional sphere of these animals. A recent scientific publication resulting from the Techcare project (Integrating innovative TECHnologies along the value Chain to improve small ruminant welfARE management; https://techcare-project.eu/) identified a number of priorities in terms of critical animal welfare issues in small ruminant production, together with some PLF technologies that can monitor the categories of welfare indicators within which these critical issues have been placed (Morgan-Davies et al., 2024). However, rather than monitoring emotional states, the indicator categories relate to conditions/states that can potentially affect the welfare of sheep and goats (e.g., weight loss or change in body condition, behavioural changes, milk yield and quality, and environmental indicators; Morgan-Davies et al., 2024).

The continuous and non-invasive monitoring of emotions using PLF technologies is very new and the available scientific publications are very limited (Neethirajan et al., 2021). Currently, research is focused on automating the collection and decoding of data/information related to individual emotional indicators, although the integrated monitoring of different indicators through the use of multiple sensors is the future goal for a more accurate assessment of the animal's emotional state (Neethirajan et al., 2021). Although precision livestock farming is still in its infancy, a variety of sensor-based solutions are already potentially available to monitor animals' emotions (Neethirajan et al., 2021). In particular, the use of technology to detect temperature (thermal imaging), electrical activity of the circulatory system (electrocardiography; ECG), heart rate variability (HRV), electroencephalography (EEG), respiratory rate, facial expressions and vocalisations seems promising (Neethirajan et al., 2021). The sensors used to detect these indicators could be combined with those used to track animals to enable the detection of individuals in a potential emergency situation (Neethirajan et al., 2021).

According to Neethirajan et al. (2021), vocalisations and facial expressions are among the most promising behavioural indicators of emotion that could potentially be recorded using integrated animal sensing systems. These two indicators are the only ones mentioned in the few scientific publications on the use of PLF technologies related to the development of systems for real-time monitoring of the emotional states of farm animals. Four studies concern vocalisations (Gavojdian et al., 2024 in cattle, Herborn et al., 2020 in chickens, Jakovljevic et al., 2019 in broilers, Ntalampiras et al., 2023 in goats) and three studies concern facial expressions (Neethirajan, 2021 in cows and pigs, McLennan and Mahmoud, 2019 e Noor et al., 2022 in sheep). Most of these studies focus on negative affective states, such as stress (Gavojdian et al., 2024; Herborn et al., 2020; Jakovljevic et al., 2019) and pain (McLennan and Mahmoud, 2019; Noor et al., 2022), while two studies examined the expression of both negative and positive affective states (Neethirajan, 2021; Ntalampiras et al., 2023). As can be seen from these publications, only three relate to PLF technologies developed to monitor the affective states of small ruminants, all using off-animal sensors (table 5).

Table 5. Technologies and Artificial Intelligence techniques adopted in PLF solutions for small ruminants

Species	Sensor	Technology	Detected parameter	Device	Computer Science technique	Reference
Goats	SensorTile sensor	microphone	vocalisations	environmental sensor	neural network	Ntalampiras et al. (2023)
Sheep	not specified: probably a photo camera	optical Imaging	facial expressions	not specified	support vector machines e neural networks	McLennan and Mahmoud (2019)
Sheep	none (images dataset from internet)	-	facial expressions	-	convolutional neural networks (CNN)	Noor et al. (2022)

The publication by Ntalampiras et al. (2023) is one of the outputs of the VOCAPRA project (Approccio multidisciplinare per la messa a punto di un sistema di monitoraggio continuo in allevamenti caprini da latte mediante analisi delle vocalizzazioni; https://vocapra.lim.di.unimi.it/), funded by Lombardy region (operazione 16.1.01 del Programma di Sviluppo Rurale 2014 -2020). The project aimed at identifying the characteristics of vocalisations emitted by goats in different emission contexts, in order to create an IT tool (smartphone application) that can be used by farmers for the continuous and non-invasive monitoring of their dairy goats. This tool has been developed to enable farmers to respond more easily to the needs of their animals by understanding their signals in a direct way, and is the first tool developed for this purpose in the small ruminant sector.

The VOCAPRA project involved four dairy goat farms in Italy, Lombardy region, where goat vocalisations were recorded continuously for over a year using environmental sensors. In order to manually label vocalisations according to the context in which they were emitted, it was essential to develop filters to isolate bleats from the large number of sounds collected (10 million WAV files with an average duration of 20 s), using decision trees (Random Forest classifiers) and transfer learning techniques (in particular YAMNet). Details of this first phase of the project and the potential use of vocalisations to monitor the emotional state of goats are given in section 5.1 "An integrated system for acoustic monitoring of goat farms". Further details of the VOCAPRA project are given in section 6 "General conclusions".

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4. Aims

The overall aim of this thesis is to increase the human knowledge of how small ruminants express their emotions, focusing in particular on acoustic parameters as animal-based indicators of affective states. To achieve this goal, in this thesis we adopted a multidisciplinary approach, ranging from the study of the direct perception and interpretation of vocalisations by humans to the use of traditional acoustic analysis to decipher the emotional content of vocalisations, through the extraction of acoustic features and the application of statistical models, and their practical application for the development of up-to-date Precision Livestock Farming (PLF) technologies. A better understanding of the acoustic signals emitted by sheep and goats, along with other indicators of emotions, will have practical implications in terms of improving the human-animal relationship and animal welfare on farm.

5. EXPERIMENTS

5.1 AN INTEGRATED SYSTEM FOR THE ACOUSTIC MONITORING OF GOAT FARMS

Ntalampiras, S., Ludovico, L. A., Presti, G., Vena, M. V., Fantini, D., Ogel, T., Celozzi, S., Battini, M., & Mattiello, S. (2023)

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1. Introduction

During the last decades, there has been a constantly increasing public interest in animal welfare, and therefore farmers are asked to guarantee increasingly high welfare standards in order to meet consumers' expectations (Cornish et al., 2016; Misra et al., 2022). Animals are sentient beings and, to ensure their welfare, we should not only guarantee that they are not suffering nor experiencing pain and provide them with appropriate housing, feeding, and management, but we should also take care of their mental state in terms of moods and feelings, for example avoiding to create fearful situations or emotional distress and respecting species-specific behavioral needs (Brambell, 1965). In this respect, the establishment and maintenance of a good human-animal relationship (HAR) may play a fundamental role (Hemsworth, 2003) as highlighted also for goats in a recent review (Celozzi et al., 2022).

Establishing positive HAR is based on the ability of humans in understanding signals emitted by animals and in the awareness of how animals react in response to human behavior (Nawroth, 2017). Communication, either visual, olfactory, tactile, acoustic, or gustative, plays a fundamental role in allowing information sharing (Immelmann, 1980). As to acoustic communication, in animals, it is used to transmit information about several specific situations (e.g. alarm, location of food sources, reproductive status, social status, etc.) and the characteristics of acoustic signals depend on specific emotional states and on the level of arousal. In general, animal vocalizations associated with positive emotional states are characterized by short duration and low fundamental frequency, even though a considerable variation has been observed regarding this last parameter; in situations of high arousal, vocalizations are usually louder and harsher while presenting a longer duration, with generally higher frequencies (Briefer, 2012). Recent studies confirm that humans are able to interpret acoustic parameters of vocalizations related to different emotional states (Filippi et al., 2017). However, so far few studies have been carried out in farm species on interspecific vocal communication aimed at assessing whether and to what extent humans appropriately recognize the emotional valence and the level of arousal of animals' vocalizations and can identify the context in which these vocalizations are emitted. Most of these studies have been conducted in pigs, whereas information on ruminant species is scarce (Briefer, 2012) and often lacks the use of advanced analysis techniques. In goats (Siebert et al., 2011) investigated some vocal traits (duration, peak frequency, pureness, and entropy) of sounds emitted in a distress situation in response to isolation, whereas a comparison of vocalizations in contexts characterized by different valence and levels of arousal was successfully carried out by Briefer et al. (Briefer et al., 2015).

In cattle, there is a limited amount of papers focusing on basic sound analysis (i.e. calves' vocalizations in response to milk deprivation or beef cattle vocalizations in response to restraining and hot iron branding) (Johnsen et al., 2018; Thomas et al., 2001; Watts and Stookey, 1999). More recently, a preliminary study on the relationship between acoustic features and animal behavior suggests the use of sound analysis to monitor dairy cattle welfare on farms (Meen et al., 2015). These preliminary works seem to confirm the earlier suggestion that the vocalization of farm animals can be successfully used to monitor animal welfare (Manteuffel et al., 2004), and support the importance of further research in this field, especially on ruminants, using recent methodologies that allow a more rigorous and detailed sound analysis (Acconcjaioco and Ntalampiras, 2021; Ntalampiras and Potamitis, 2021; Stowell, 2017). In fact, according to a recent review (Laurijs et al., 2021), no automated measurement system is currently available for identifying the emotions of farm animals based on their vocalizations, let alone the use of smartphone applications facilitating the communication of such information.

This article presents the work carried out within the ongoing project entitled "Approccio multidisciplinare per la messa a punto di un sistema di monitoraggio continuo in allevamenti caprini da latte mediante analisi delle vocalizzazioni" (VOCAPRA)[1], aiming at capturing, processing, and modeling goat vocalizations in farm environments. There is an urgent need to make available to farmers knowledge and tools that enable them to respond efficiently to problems that can occur on the farm. To this end, the VOCAPRA project proposes to use a sensor network to monitor the farm environment and automatically characterize the captured information. The project belongs to the scientific area of Precision Livestock Farming (PLF) which researches intelligent management and care of animals in livestock farming via making informed decisions. More specifically, PLF uses automatic monitoring systems to not only optimize production and/or reproduction but also to improve animal health and welfare (Gómez et al., 2021; Niloofar et al., 2021).

This work moves from controlled laboratory conditions to real-world ones where a great amount of diverse problems needs to be solved including highly non-stationary noise, unpredictable interference of diverse types of sound events non including goat vocalizations, weather conditions, preserving the privacy of the working personnel, etc. The novel points of the proposed monitoring system are the following:

- it is comprised of a multimodal hybrid wired-wireless sensor network,
- it operates 24/7 and has been tested on data recorded over the duration of one year,

[1] https://vocapra.lim.di.unimi.it/index.php

- it preserves the privacy of the working personnel by excluding every non-goat vocalization sound event,
- it can provide an accurate classification of the existing goat vocalizations and notify the authorized personnel via a suitable smartphone application.

This would open new opportunities to develop up-to-date real-time automatic monitoring and management systems of goat farms based on vocalization analysis, which would increase the range of already available PLF tools.

The project is divided into four partially overlapping phases:

1. collection of basic data through a one-year-long data harvesting campaign, using dedicated devices (cameras and microphones) in the partner farms;
2. manual labeling of the most relevant events by a team of animal scientists, so as to complete our ground truth;
3. training and testing the models upon the aforesaid dataset;
4. implementation and testing in real conditions of a prototype of the proposed system.

At the time of writing, phase 1 is complete, phases 2 and 3 are at a final stage, and phase 4 is starting.

2. The goat farms

The project involves four dairy goat farms with different characteristics situated in different provinces of the Lombardy region (Northern Italy).

Farm A, located in Lecco province, is a semi-intensive organic certified farm with 22 ha of forage, pasture, and cereals, where animals have daily access to pasture during the morning in the hot season (from 10 a.m. to 12 a.m.). It breeds about 105 lactating Alpine goats, housed in a 29.1 × 16.0 m barn divided into 3 pens. Sensors for the collection of goat vocalizations were installed only in two of these pens (five sensors, three in one box and two in the other one, along the long side of the farm). In the first days of testing, goat kids were housed in the same barn with adult goats and they were allowed to physically join their mothers from 5 a.m. until early afternoon to suckle. After weaning, goat kids were moved to another barn. Goats were milked twice a day, at 5:30 a.m. and 4:00 p.m. in a milking parlor situated in a building next to the main barn (duration of milking: approximately one and a half hours). Animals were fed with hay (4 times/day) and concentrate (thrice/day). Farm B is located about 600 m above sea level in the Oltrepo’ Pavese hills (Pavia province). It is a family-run conventional intensive farm with about 64 lactating Alpine goats. All adult goats are housed in a single pen in a barn of 27 × 10 m. Four sensors were installed on the long side of the barn. Animals have access to pasture during the morning (from 9:00 a.m. to 11.30 a.m.) in the hot season. Goat kids are housed in a separate pen within the same barn. Goats are milked twice/day at 6:30 a.m. and 6:00 p.m. (duration of milking: approximately one and a half hours). Goats are bred during the natural breeding season (October–November), give birth in spring (February–March), and produce milk from parturition until the dry period (two months before the next parturition). However, 16 goats are milked all year round (long lactation), with no dry period between births. Milking takes place in a separate room located in the same barn where goats are housed. Goats are fed with forage (thrice/day) and concentrate (4 times/day).

In Farm C, approximately 95 lactating Saanen goats are bred in a conventional and intensive way. They are housed in five pens located in a recently-built large barn (40.5 × 18.6 m). Environmental enrichments are present in each pen (rocks to climb and large gum balls), in order to improve goats' welfare. A sensor was installed on one side of each pen. Kids are housed in the same barn as adult goats but in separate pens. Goats are milked twice a day (6 a.m. and 6 p.m.) in a milking parlor separated from the barn. They are fed with hay (once/day) and concentrate (twice/day).

Farm D is a conventional intensive farm belonging to the University of Milan, used mainly for educational and experimental purposes. It hosts 29 Alpine goats, housed in a 25 × 12 m barn and divided into six pens, four of which were involved in the present study. In this farm, from May to October during daylight hours each pen has access to an outdoor area, which provides extra space and a more diversified and stimulating environment. Two sensors were installed inside the barn and two in the external area, in order to monitor goats also when they were in the outdoor yard. Goat kids are separated from their mothers immediately after birth and housed in a pen in the same barn as adult goats. Milking is performed once a day around 8 a.m. in the same barn. Feeding management varied during the experimental period because a feeding trial was ongoing at the same time as the present study.

3. The wireless acoustic sensor network

The block diagram of the constructed monitoring system is shown in Fig. 1. The rationale behind the designed system architecture is to cover areas where events of interest are going to occur. As a first step, animal scientists carefully selected the areas within each farm that are of interest meaning that relevant activity is expected to take place. Then, considering the surface which is required to cover along with the possibilities of installing the required devices, the number of acoustic sensors and their exact topology were decided. These were placed in positions chosen by animal scientists in places where significant acoustic activity is expected to occur.

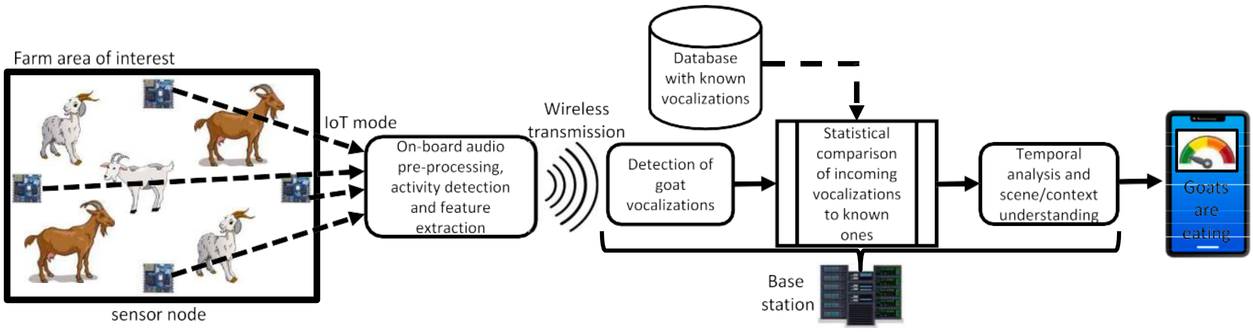

Fig. 1. The block diagram of the proposed monitoring system: starting from IoT nodes, where audio pre-processing, activity detection and feature extraction are carried out, the system transmits the data to the base station for further analysis and scene understanding. Finally, the prediction is made available to the end user via a smartphone application.

A characteristic example is demonstrated in Fig. 2. Incoming audio was captured by STMicroelectronics SensorTile sensors[2] integrating state-of-the-art processor and sensing system facilitating IoT applications.

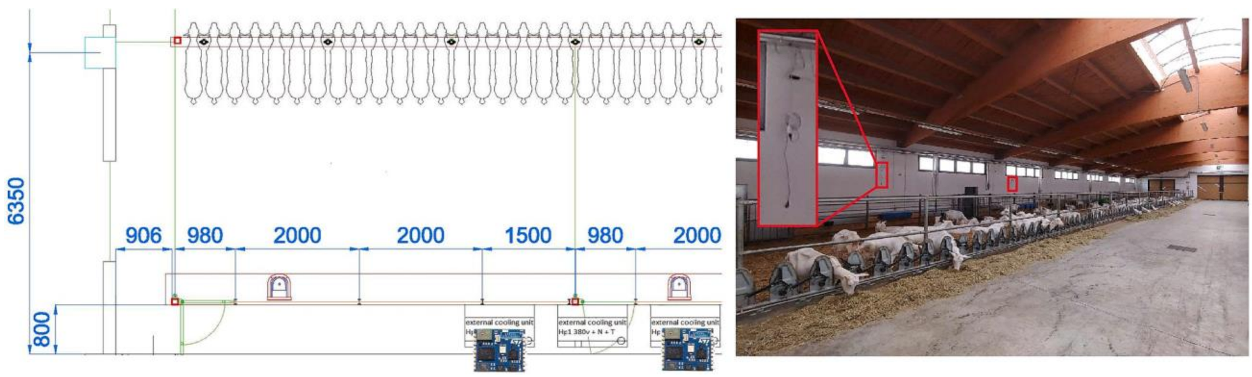

Fig. 2. A characteristic example of positioning the Sensortiles in IoT mode. We see an area of interest covered by two sensors shown in red rectangles in the picture (measurements are in cm).

A close-up picture of a Sensortile is shown in Fig. 3. As such a wireless acoustic sensor network (WASN) was formed for each farm composed of a different number of sensors depending on the characteristics of each area of interest. The number of acoustic sensors per farm is provided in Section 2. Such sensors were chosen because they have the CPU capability to preprocess the incoming data and filter out parts without acoustic activity. At the same time, they have considerable memory capacity to facilitate the aim of the project, i.e. long-term monitoring of goat farms. After deciding the topology and installing the sensors, the WASN operates as follows: a) the sensor continuously executes an acoustic activity detection algorithm filtering out silent parts,

[2] https://www.st.com/en/evaluation-tools/steval-stlkt01v1.html

b) a subsequent algorithm detects goat vocalizations by discarding all non-goat activities, e.g. environmental noise, human speech, etc. These processing stages are explained in the following subsections.

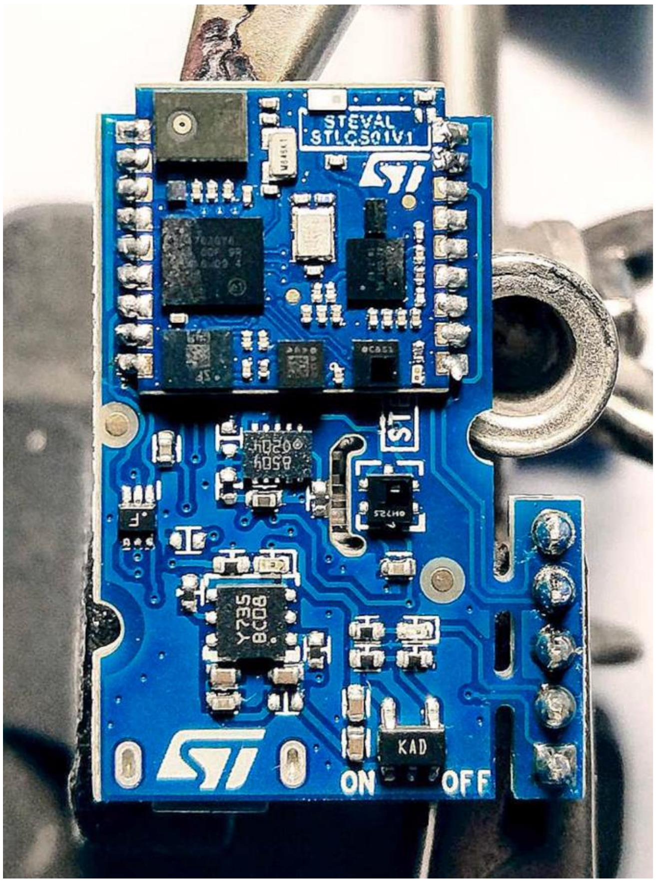

Fig. 3. A close-up picture of a Sensortile.

3.1. Acoustic activity detection

Acoustic Activity Detection (AAD) was performed by means of sensors programmed to record sound events exceeding an adaptive threshold. A characteristic example of positioning the sensors in IoT mode is shown in Fig. 2. The state diagram of the AAD is shown in Fig. 4.

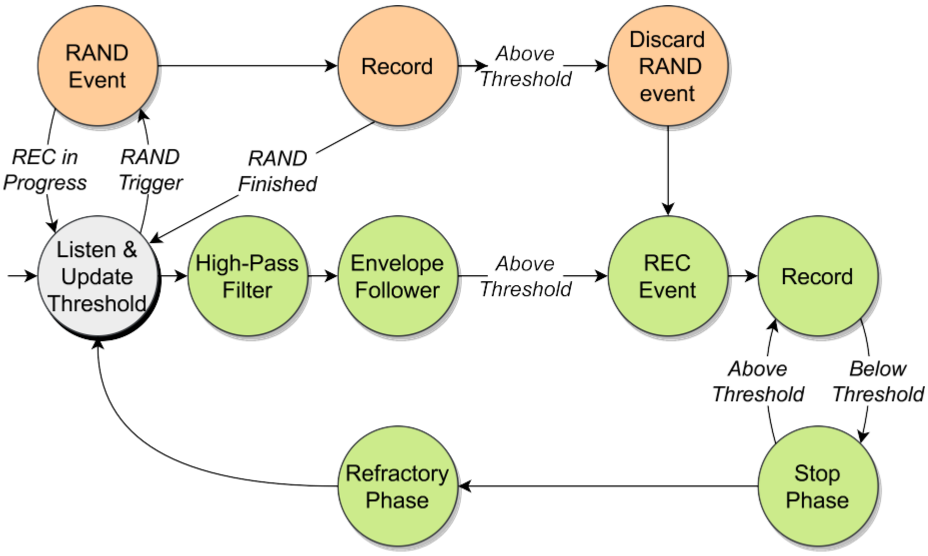

Fig. 4. State diagram of the Acoustic Activity Detection (AAD).

The sensor is constantly listening through its microphone with 16 kHz of sample rate and 16 bits as bit depth. The captured sound signal is stored and processed with a 16-sample long buffer. The sound signal passes through a biquadratic high-pass filter (cutoff frequency: 200 Hz; Q: 0.707). Then, an envelope follower based on Root Mean Square (RMS) is applied to the filtered signal. While listening, the SensorTile checks if the envelope e exceeds the threshold τ multiplied by a delta value δ. In this case, a REC event is triggered and the sound recording starts. A buffer is exploited to record the audio signal 1 s previous to the REC event trigger. When a REC event is in progress and the envelope e falls below $\tau \cdot \delta$, the device enters the stop phase and continues the recording for 5 s. If e exceeds again $\tau \cdot \delta$ within the stop phase, the recording resumes. Otherwise, after 5 s, the device enters the refractory phase in which no REC event can be triggered. Then, the device resumes listening when the recording of the audio samples in the queue has been completed.

The threshold τ is constantly updated to be adapted to the environment's loudness. In particular, the threshold is set to an initial value τ_0, then the threshold τ_n at time *n* is so computed:

$$\tau_n = \begin{cases} \tau_{n-1} + \lambda^{+}(e - \tau_{n-1}), & \text{if } e > \tau_{n-1} \\ \tau_{n-1} + \lambda^{-}(e - \tau_{n-1}), & \text{otherwise} \end{cases}, \tag{1a}$$

$$\tau_n \leftarrow \begin{cases} \tau_{min}, & \text{if } \tau_n < \tau_{min} \\ \tau_{max}, & \text{if } \tau_n > \tau_{max} \\ \tau_n, & \text{otherwise} \end{cases}, \tag{1b}$$

where $\lambda^+ = 8 \times 10^{-6}$ and $\lambda^- = 1 \times 10^{-5}$ control the influence of the envelope e on the threshold increase and decrease, respectively. Then, in Eq. (1b) the threshold is limited by upper and lower bounds τ_{min} and τ_{max}, respectively.

To collect background noise samples, the SensorTile generates 10 RAND events per day lasting 5 s each. If the envelope e exceeds $\tau \cdot \delta$ while a RAND event is in progress, the RAND event is discarded and a REC event is triggered. While a REC event is in progress, no RAND event is generated and if there's a scheduled RAND event it will be postponed at the end of the REC event.

3.2. Detection of goat vocalizations

During the initial phase of the recording campaigns, it emerged that the number of sound events occurring on the farms is enormous, thus the quantity of the acquired data is massive. This would pose a significant burden for the labeling team which had to manually annotate data, the majority of which did not include goat vocalizations. To this end, we developed a detection algorithm for distinguishing goat vocalizations from all other sounds. As such, the aim here is to filter out data that do not include goat vocalizations so that subsequent processing phases are focused only on those.

The initial dataset was created through the manual annotation conducted by animal scientists. This resulted in a corpus containing the following 5 classes:

- *Goats:* various types of goat vocalizations,
- *Milking Machine:* sounds emitted by the milking machine,
- *Machinery:* sounds associated with other machinery present on the farms,
- *People:* sounds associated with human activities, e.g. speech, laughing, clapping, etc., and
- *Noise:* various types of environmental noises.

This initial dataset includes approximately 1 h and 7 min of sounds distributed in a balanced way across the above-mentioned classes. Care was taken so that audio files categorized as goats' vocalizations included only bleats so that the detection algorithm elaborates on the specific audio structure alone. After constructing the initial dataset, we developed two solutions for detecting goat vocalizations: a) one based on handcrafted features and a traditional machine learning algorithm, and b) one relying on the deep network YAMNet designed to facilitate generalized sound recognition applications.

3.2.1. Traditional machine learning

In the first algorithm, the detection of goat vocalizations is based on a wide variety of temporal and spectral audio features (Mel-Frequency Cepstral Coefficients, Root Mean Square, Spectral Centroid, Spectral Bandwidth, Spectral Rolloff, and Zero crossing rate (Alas et al., 2016; Ntalampiras et al., 2020) which, after early experimentations, have proven to be useful in characterizing the available audio classes[3]. These are fed to a Random Forest (RF) classifier learning rules associating the input features to the final classes.

Since the available files are of different lengths, we employed a sliding window with an overlap of 50% so that it was possible to divide the available audio into fragments of the same size during both training and testing. The overlap was required in order not to lose potentially important information existing on the edges of the window. On top of that, there are a plethora of cases where more than one classes exist in a single file as outputted by the AAD algorithm. The highest performance was obtained with a sliding window lasting 2 s. As regards the RF, the number of trees was set equal to 500 after an optimization phase consisting of a grid search within the set {5,10,25,50,100,500,1000}. Interestingly, the algorithm's predictions are used to count the number of bleatings existing in a specific audio file towards characterizing their activities.

3.2.2. Transfer learning based on YAMNet

The second algorithm exploits the representation extracted from a deep neural network, i.e. YAMNet, which is pre-trained on data coming from 521 classes. Interestingly, YAMNet follows the MobileV1 convolution architecture and is trained on the AudioSet-YouTube corpus (Shor et al., 2020). Towards using YAMNet, the audio data is processed as follows:

1. The audio is converted to a monophonic channel and resampled at 16 kHz.
2. The spectrogram is extracted and created using STFT magnitudes of 25 ms overlapped by 15 ms.
3. The Mel spectrogram is calculated by mapping to 64 bins of the Mel scale within the range of 125–7500 Hz.
4. Finally, the algorithm is applied and its outcome is passed to YAMNet model for extracting the embeddings.

[3] The librosa python package available at https://librosa. org/doc/latest/index.html was employed at this stage

These are pulled from YAMNet and then fed as input to a sequential and a reduce mean layer mapping to the classes mentioned above (Ntalampiras et al., 2021). At this point, the final layers of the network map the YAMNet embeddings to the available labels as shown in Fig. 5.

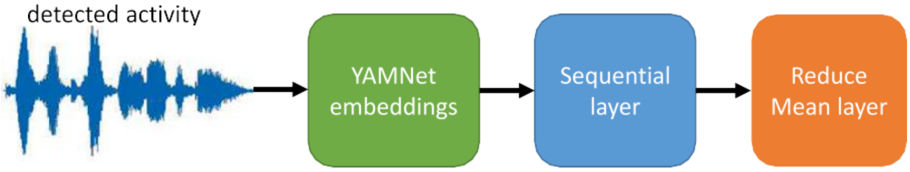

Fig. 5. Block diagram of the goat vocalization algorithm based on YAMNet embeddings.

3.3. Results on goat vocalization detection

The dataset presented in Section 3.2 was divided into 70% for training, 10% for validation, and 20% for testing purposes. The division was kept the same when experimenting with RF and YAMNet-based classifiers in order to achieve a reliable comparison. RF classified correctly the goat vocalizations 75% of the time, while YAMNet reached 98.1%. As our main focus is to collect goat vocalizations for further processing and interpretation, we concentrate on YAMNet's performance, which demonstrated promising performance in detecting such sound events and discriminating them from the rest of the classes. The confusion matrix is presented in Table 1. We observe several errors in Farm C where loudspeakers are installed and play various types of music, which is supposed to have a relaxing effect on goats and to positively contribute to their welfare (do Nascimento et al., 2022). In fact, the errors were associated with music which includes female sung parts. Other errors were associated with the existing highly non-stationary background noise. In addition, most of the misclassifications concern machinery and milking machine classes which are expected up to a certain extent due to the acoustic similarities of these two classes. However, it is not relevant since we aim at detecting goat vocalizations.

Thus, the YAMNet-based classification algorithm was immediately put into production to assist in the finer annotation of the detected vocalizations as described in the following section. At the same time, the present algorithm is updated on a regular basis with new data as extracted by the clustering process described in Section 4.3 in order to further improve its performance.

Table 1. Confusion matrix obtained using the YAMNet-based classifier (%). The highest rates are emboldened.

Presented/Predicted	*Goat*	*Machinery*	*Milk. Mach.*	*Noise*	*People*
Goat	**98.1**	–	–	1.3	0.6
Machinery	–	**61.9**	23.8	9.5	4.8
Milking machine	–	6.3	**81.3**	12.5	–
Noise	–	1.6	0.8	**97.6**	–
People	2.2	–	–	–	**97.8**

3.4. The smartphone application

The smartphone application (see Fig. 6) was designed keeping in mind the requirements of the two main users within the farm, i.e. the Head of Farm (HF) who manages the farm from an organizational and sales point of view, and the Stockperson (S) who deals with the management of the animals (feeding, cleaning, milking). Such users have extremely different needs: HF is interested in the average welfare of the animals as she/he is a more "managerial" figure than S, who is instead interested more in specific "real-time" monitoring since working directly with animals needs instant notifications in order to be able to intervene promptly in case of need.

Therefore, the smartphone application was developed taking into account such versatility of necessities within the farm:

1. display of graphs and statistical descriptors that show the HF the well-being of the animals
2. a notification system that allows S to intervene immediately in case of need.

Furthermore, given the relatively large set of labels, it is possible for both HF and S to select a subgroup for which they wish to be notified. The application includes two main pages, each one adapted to each farm user. More in detail, the page dedicated to HF: 1. allows the user to monitor the farm through a graph of the occurrence of the various activities/events defined as "interesting", 2. a daily occurrence counter, 3. a correctly classified occurrence counter and finally, the average time response of F to notifications.

The page designed for S includes a checklist of activities that are currently occurring on the farm. A single activity in the list is composed of the following: 1. each occurrence is associated with a name, 2. a "notification viewed" indicator that allows S to know if she/he has already intervened to manage the alert, 3. an option that allows S to select the activities marked as "viewed" and 4. a form where S can indicate the correct prediction in case a model misclassification occurs. Such functionality is expected to assist in gathering data in order to improve the classification algorithm in an online mode.

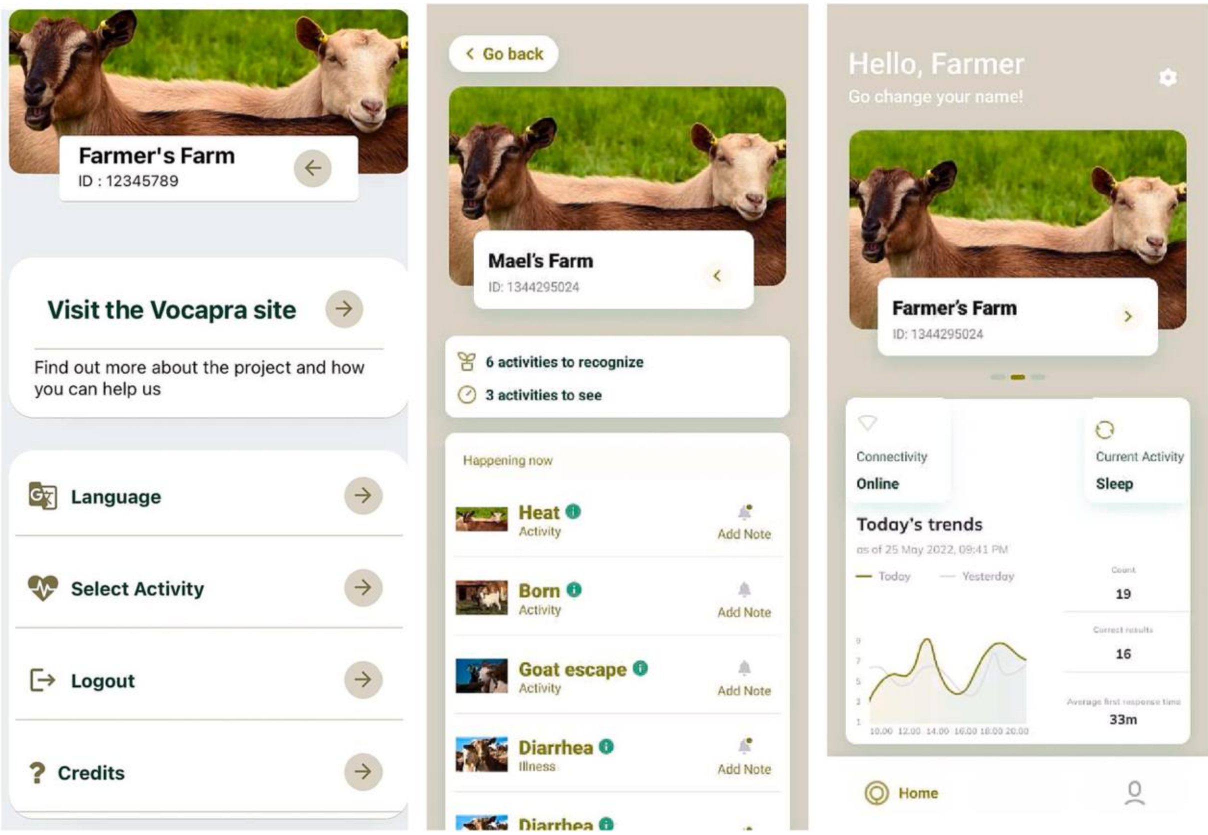

Fig. 6. Left to right: a) settings screen, where the events/activities of interest can be selected; b) page dedicated to F, where she/he can see the list of all interesting events/activities; there is the option to correct an erroneous system prediction; c) page dedicated to HF with the possibility to consult a daily graph of activities divided by class. The option to access the specific notifications screen is included.

4. Dataset overview, annotation, and exploration

This section a) provides an overview of the dataset obtained so far, b) analyses the results of goat vocalization detection, c) describes the data annotation process, and d) presents the results of clustering the available goat vocalizations.

4.1. Dataset overview

The current state of the dataset recorded as of June 2022 is tabulated in Table 2. There, we see the number of annotated events and the number of audio files along with their duration per farm. Even though the recording campaign started at the same time (April 2021), the number of files differs between the various farms depending on the existing acoustic activity. Furthermore, it depends on the number of goats present on each farm. Goats are seasonal breeders, and this affects their behavior throughout the year (de la Lama and Mattiello, 2010).

Table 2. The current state of the dataset at the end of June 2022.

Farm	# Annotated Events	# Audio Files	Duration (h)
A	1046	3,468,415	~10,527.82
B	791	2,939,211	~12,519.97
C	961	1,662,819	~5036.06
D	2171	1,920,752	~6172.18
Total	4969	9,991,197	~34,256.03

Therefore, the recording campaign was planned to last an entire year so as to capture the variations that goat vocalizations demonstrate throughout the year. Overall, we have collected approximately 10 M sound events lasting more than 34,000 h. Fig. 7 illustrates the data distribution per farm in terms of number of audio files, annotations and durations.

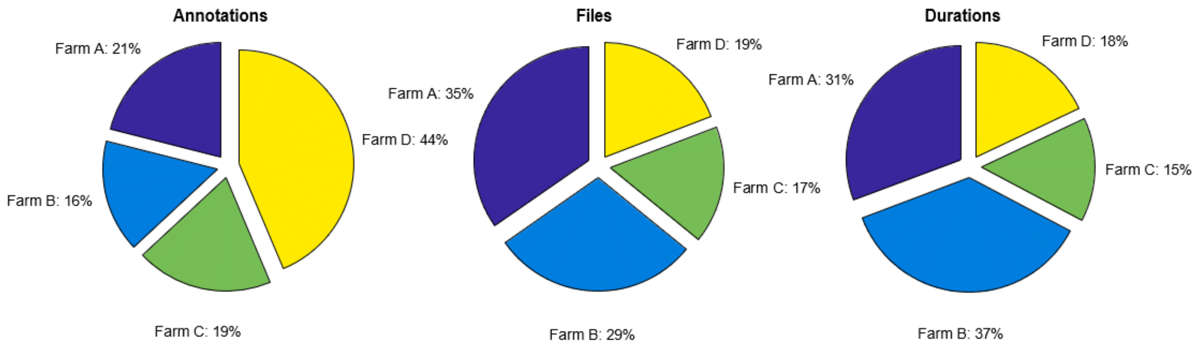

Fig. 7. Data distribution per farm in terms of number of audio files, annotations and durations.

Fig. 8 illustrates a hierarchical organization of the available classes. The first level includes an emotional characterization, i.e. pain, stress, or joy/excitement, while the second level specifies the subclasses and lists several examples. It is worth noting that the same type of subclass may be associated with positive or negative emotions depending on the case; for example interaction with another goat can be negative (e.g. competition) or positive (simultaneous presence of mothers and kids).

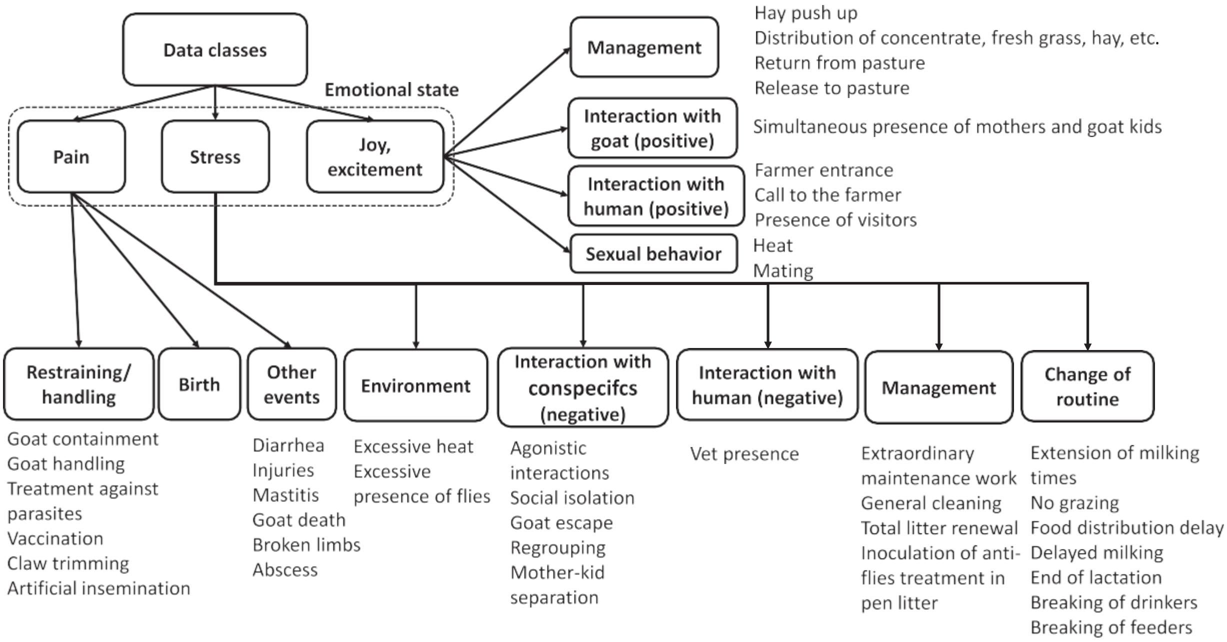

Fig. 8. Hierarchical organization of the classes existing in the so-far annotated dataset. The first stage provides an emotional classification, while the second one subclasses with examples. The variables used in the study (i.e. contexts of emission) are those out of boxes and are organized in classes (pain, stress, joy, excitement) and subclasses, e.g. restraining/handling.

4.2. Classification-assisted annotation of the detected goat vocalizations

The annotation process is based on the information provided by the available farm records filled by the stock persons and the output of the YAMNet-based goat vocalization detection algorithm described in Section 3.3. Furthermore, cameras were positioned in the barns, and videos were transmitted via WiFi to a digital video recorder and stored on a hard disk. These videos, together with farm records, were used to support animal scientists in the labeling of goat vocalizations. To match the registers and domain expert knowledge, we designed and developed a web-based annotation tool available in the back-end area of the official project's Web site (https://vocapra.lim.di.unimi.it/). The list of possible events (i.e., event types) was provided by domain experts, namely animal scientists and farmers. Fig. 8 illustrates the variables used in the study (i.e. contexts of emission) are those out of boxes and are organized in classes (pain, stress, joy, excitement) and subclasses, e.g. restraining/ handling. Event instances are represented by timestamped occurrences of a given event type.

Recurrent instances and/or human-driven instances (food distribution, milking, livestock handling, etc.) are made automatically available in the digital version of farm records; nevertheless, unpredictable instances must be manually added a-posteriori.

Essentially, the web tool allows authenticated users to browse, select and listen to goat vocalizations, compile, or revise farm records, and, as a result, obtain audio files tagged on the basis of farm events. The browser app offers several features to ease such a task. For example, together with audio files, an ad-hoc web page shows the continuous video recording taken from the previously described cameras suitably positioned in the barns. The synchronized performance of audio files and video footage should help recognize events that have not been listed in the log. These integrations are particularly relevant in case of unexpected events, such as injuries, visiting strangers, animal interactions, storms, the presence of dogs, etc. Another feature of the web interface is the possibility to have a short list of the most relevant audio files in a day. The relevance is automatically computed by an algorithm (see Section 3.2) that parses audio files and returns the probability of bleating presence with respect to the soundscape and other non-relevant background sounds. In this way, if the farm record needs to be manually integrated, namely if no event occurrence has been already matched with a relevant audio file, the domain expert can first address such cases. The annotations are saved into an SQL database. The database management system in use is PostgreSQL version 9.2.24. The main entities contained in the database are event types, event instances, and audio files. Event types and event instances are in a one-to-many relationship; examples of event types include “goat birth”, “goat death”, “food distribution”, etc. The occurrence of a given event type can be periodical (with a daily or yearly frequency), sporadic, or unpredictable. Event instances are, for example, the birth or death of a goat or the distribution of fresh grass occurring in a given farm, at a given date and time.

Audio files are of variable length, since the recording was not continuous but triggered and turned off based on the sound levels detected by the sensors. Sound tracks were saved onto a NAS (network attached storage) connected to the application server, which made them available to the database and the web interface. Consequently, as regards audio files, the database provides only metadata and pointers to the digital objects on disk. From a logical point of view, audio files and event instances are in a many-to-many relationship: an audio file could cover, either partially or completely, 0 to n event instances; and, vice versa, a single event instance could be connected to 0 to m audio files. This relationship is not explicit in the database (e.g., through a dedicated table); it is reconstructed based on the start times and durations of both entities. A particular scenario is that of event instances whose duration is very short (impulsive events, duration $d \approx 0s$) or impossible to detect (unknown events, duration d = - 1). In these cases, the event is automatically associated with all the audio files that cover its start time.

It should be noted that the web annotation tool does not actively intervene in audio files, being only a viewer/player for them. Conversely, by listening to audio files (and watching the corresponding video footage), it drives the user to enter additional farm events, i.e. instances, and possibly correct old ones. In this way, event definitions are intrinsically converted into labels associated with audio files thanks to the time relationships described above. Last but not least, given the fact that it is not feasible to manually annotate all acquired data, days are prioritized based on the expected goat vocalizations using the information existing in the farm registers.

4.3. Clustering of goat vocalizations

In order to refine the ML models, a clean subset of labeled vocalizations without any background noise was generated as follows.

We selected the top 2000 files based on the score given by the bleating detector of Section 3.2 that had already been labeled by experts. All these files were carefully listened to so as to ensure that bleatings were clearly distinguishable from any type of background noise. This resulted in the selection of 273 files containing at least one bleating.

Each file containing bleatings was split into segments via an automatic segmentation algorithm (Haus et al., 2017). This algorithm is based on the hierarchical clustering of small audio frames using Mel- Frequency Cepstral Coefficients (MFCCs) (Ntalampiras et al., 2019) and the Euclidean distance as metrics. In particular, the resulting dendrograms were cut so as to obtain about 4 clusters of segments per sound file; this threshold was chosen in order to have the audio signal split into different classes, with at least one of them corresponding to bleatings only, and to have segments at least 250 ms long to avoid too short segments.

The segments were then hierarchically clustered again, with the clustering features being the MFCCs averaged across time. This time the dendrogram was cut to have only 2 clusters as follows: one composed of bleatings only, while the other composed of all other sounds. This was possible because of the strong similarity among bleatings compared to the great variety of other noises present in farm environments.

The non-bleating cluster was discarded, and the bleatings one is the output of the process; there, 2431 files were marked as single bleatings and employed to refine the goat vocalization detection algorithm (Section 3.2).

Using this part of the dataset, a preliminary statistical analysis was carried out to investigate the existence of differences among classes shown in Fig. 8. Nevertheless, it should be mentioned that this analysis does not consider all labels, since it has been conducted only on a subset of the entire dataset. As such, it is not exhaustive, while a systematic statistical analysis is left for future work.

To this end, the following generic acoustic features (Alas et al., 2016) were extracted from each bleating, i.e. a) pitch, P (log(Hz) values); b) envelope, E (dB_{fs}); c) spectral centroid, C (log(Hz)); d) roughness, R (amount of rapid amplitude variations (Daniel and Weber, 1997)); e) duration, T (seconds). Then, for each time-varying feature, we calculated the mean value ($\cdot_m$), and the mean delta ($\cdot_{dm}$) resulting to a dimensionality equal to 9.

Finally, we collectively considered goat vocalizations recorded in all four farms and conducted a statistical analysis based on the Kruskal-Wallis test since the available data is not normally distributed. Interestingly, we discovered statistically significant differences among several of the classes presented in Fig. 8. Post-hoc pairwise comparisons were carried out for features that resulted significantly different (p <0.001), i.e. P_m, E_m, E_{dm} C_m, R_m, and T. Among others, the following observations emerged:

1. Differences were found between medians of feed distribution and daily routine operations (P_m = 54.84 vs 62.07; E_m = - 27.74 vs - 32.75; C_m = 93.24 vs 96.13; R_m = 4.18 vs 3.04, respectively; all p <0.001).
2. Bleatings emitted during mother-kid separation had significantly higher R_m and E_m, and lower P_m, C_m and E_{dm} than those emitted during daily routine operations (R_m = 4.23 vs 3.04; E_m = - 25.15 vs - 32.75; P_m = 59.83 vs 62.07; C_m = 93.54 vs 96.13; E_{dm} = 0.01 vs 0.03, respectively; all p < 0.001).
3. Finally, intraspecific bleatings presented significantly higher P_m and lower E_m, C_m, R_m and E_{dm} than vocalizations emitted during daily routine operations (P_m = 74.10 vs 62.07; E_m = — 38.03 vs —32.75; C_m = 93.45 vs 96.13; R_m = 2.54 vs 3.04; E_{dm} = — 0.01 vs 0.03, respectively; p < 0.001).

These preliminary results suggest that it is possible to develop a system for the automatic classification of goats' vocalizations emitted in different contexts.

5. Conclusions

This work presented the design and implementation of a WASN developed to monitor goat farms and provide an early warning system to inform farmers about their goats' emotional state. This system could be successfully implemented and possibly integrated with other PLF tools to improve farm animal welfare. The complete pipeline was explained starting from sensor placement and operation up to the smartphone application providing real-time information characterizing the farm of interest. At the same time, we provide a hierarchical organization of the involved sound classes focusing on the animals' emotional states. Importantly, the proposed architecture was successfully installed on four different farms of diverse needs and characteristics. Collective data analysis suggests the presence of statistically significant differences among the classes of interest.

However, it is important to be aware that even though PLF technologies are powerful tools to support farmers, they might present some potential threats to animal welfare (Tuyttens et al., 2022), for example due to possible technical failures, or inaccurate predictions. Furthermore, animal welfare may be posed at risk when farmers become over-reliant on such technologies, and therefore tend to lose their knowledge of animals' needs and spend less time with the animals, with negative consequences on HAR quality. For these reasons, besides the development of the mobile application which is presented in this paper, the VOCAPRA project also aims at improving the human-goat relationship by enabling farmers to better understand the meaning of vocalizations emitted by goats in different contexts, which is part of our future work. Moreover, in the future, we wish to a) create physical models analyzing goat vocalizations, b) develop suitable and interpretable classifiers for recognizing goats' emotional states, c) suitably incorporate their corresponding predictions in the smartphone application generating alerts when needed, d) explore the automatic identification of goat individuals, e) properly organize the dataset so that it can be used as didactic material for young animal scientists, students, veterinarians, technicians and farmers, and f) evaluate the farm's output under various management scenarios, where the main objective would be to minimize animals' stress levels while maintaining productivity.

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5.2 HUMAN PERCEPTION OF THE VOCAL EXPRESSION OF EMOTIONS IN GOATS

Celozzi, S., Prato-Previde, E., Battini, M., Ntalampiras, S., Ludovico, L. A., Presti, G.,

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Ready for submission to: *Agriculture and human values*

1. Introduction

In the last two decades scientific interest in the study of animal emotions and their expression has grown, leading to many publications that provide valuable insights and knowledge in this field (Boissy et al. 2007; de Waal 2011; Anderson and Adolphs 2014; de Vere and Kuczaj 2016; Laurijs et al. 2021).

The comparative approach to emotions showed that non-human animals have short term emotional reactions in response to specific internal or external stimuli perceived as relevant for survival and well-being (Mendl et al. 2010). Animals' emotional reactions are multi-componential and characterized by coordinated neurophysiological, behavioural and cognitive changes which can be investigated and measured either separately or in conjunction (Scherer 2009; Mendl et al. 2010; Panksepp 2011; Mendl and Paul 2020). It has been suggested that animals (at least mammals) may have subjective experiences (i.e., feelings) and that, in the absence of language, animals' feelings could be inferred based on behavioural, neurophysiological and cognitive indicators and responses to a specific event (de Waal 2011; Burghardt 2019; Kret et al. 2022).

Animal emotions are central to the concept of animal welfare and that understanding and assessing the valence (positive or negative) of emotional states of animals is a mean to improve their welfare and to develop more effective and humane management practices (Désiré et al. 2002; Boissy et al. 2007). Recognizing positive emotions, such as contentment and pleasure, serves as a suitable indicator of good welfare conditions, while identifying negative emotions, including stress, fear, and pain, is essential for a timely intervention to mitigate or prevent suffering (Bukhari et al. 2023).

Behavioural expression is an important indicator of emotions in both humans and animals: it is directly observable, measurable and may provide information on the individual's emotional state and its affective valence and arousal (Boissy and Lee 2014; Reimert et al. 2023). Results from behavioral studies show that domestic (e.g., cats, dogs, goats, pigs and horses) as well as non-domestic (e.g., monkeys, great apes) animals express and communicate various emotional states through body postures (eg., Reefmann et al. 2009; de Oliveira and Keeling 2018; Whittaker and Marsh 2019)), facial expressions (McLennan et al. 2016; Battini et al. 2019) and vocalizations (Briefer and McElligott 2012) and may also combine and integrate different modalities (e.g. Schnaider et al. 2022).

Vocalisations have been studied as indicators of emotions both in farmed and wild species such as pigs (Briefer et al. 2022), cattle (Schnaider et al. 2022), poultry (Marx et al. 2001), sheep (Nowak et al. 2008), goats (Briefer et al. 2015), wild boars and Przewalski's horses (Greenall et al. 2022).

The ability to recognize emotional states of other individuals plays a pivotal role in both intra- and inter-specific social interactions and relationships, allowing an individual to respond properly to others individuals (Panksepp 2009; Briefer 2018). There is evidence that positive interactions and relationships between humans and wild and domestic animals entail the capacity of both humans and animals to express their affective states and depend on the understanding of emotions and expressive behaviour of the counterpart (e.g. Hartmann et al., 2017). This is of relevance for human-animal interactions, as the misunderstanding of animals' communicative signals can compromise animal welfare and lead to injuries (e.g., dog bites, attacks, human injuries when interacting with farm animals).

The ability to understand the emotional content of vocalizations produced by other species is defined "voice-induced cross-taxa emotional recognition" (Scheumann et al. 2014). Research in this field has primarily considered humans' ability to recognize the contexts in which animals' vocalizations are produced and the emotional states conveyed by these acoustic signals.

Studies on piglets, cats, dogs, and chimpanzees suggest that humans are able to recognize negative contexts of emission better than positive contexts (Scheumann et al. 2014; Prato-Previde et al. 2020; McGrath et al. 2024). This is probably due to the fact that acoustic signals emitted in negative contexts are likely to convey information about possible dangers and are therefore more important in interspecific communication. However, the recognition accuracy of the emotional valence varies between and within animal species (Scheumann et al. 2014). Regarding the human ability to discriminate emotion-generating contexts, evidence is discordant even for species that entertain very close relationships with humans, such as companion animals (Pongrácz et al. 2005; Prato-Previde et al. 2020). More recently, (Filippi et al. 2017) and (Greenall et al. 2022) suggested that humans can better identify the emotional arousal rather than the valence from animals' vocalisations. This could be explained by the fact that human's recognition of the emotional content expressed by animal vocalisations is correlated with the vocal parameters of the sounds, and these parameters vary with arousal in a similar way across species, whereas vocal parameters of emotional valence might be more species-specific (Pongrácz et al. 2005; Greenall et al. 2022).

A number of studies suggest that factors such as previous experience with animals, gender, age and the level of empathy play an important role in deciphering the context and emotional content of animal vocalisations, even though contrasting results have been reported in some cases (Pongrácz et al. 2005; Tallet et al. 2010; Scheumann et al. 2014; Prato-Previde et al. 2020; Greenall et al. 2022; McGrath et al. 2024).

Empathy affects human's ability to interpret affective states in humans and in other species and actually, from a psychological perspective, it is referred to as the capacity to understand and share the feelings of others (either conspecific or not). It comprises both emotional and cognitive components and there is evidence that empathy for humans and empathy for animals are correlated (Paul 2000; Norring, Wikman, A.-H. Hokkanen, et al. 2014; Kujala et al. 2017; Young et al. 2018; Gómez-Leal et al. 2021). Veterinarians' empathic abilities affect their evaluation of animal pain (Norring, Wikman, A.H. Hokkanen, et al. 2014) and people scoring higher in empathy perform better in interpreting dogs' and cats' as well as different ungulates' vocalizations compared to people scoring lower in empathy (Prato-Previde et al. 2020; Greenall et al. 2022).

The study of the expression of emotions in goats (*Capra hircus*) and the investigation on how they are perceived by humans is relevant for ensuring animal welfare, as human-goat interactions take place in different contexts and situations (Celozzi et al. 2022). Goats are kept in a wide range of conditions, from intensive indoors to extensive pasture-based farming systems, from small holdings and family-run farms to industrial animal production systems (Dubeuf 2023). Moreover, in the last decades goats have been gaining increasing importance as therapeutic animals, for educational purposes, as companion animals or just for recreational activities, interacting with humans in contexts such as zoos, pet farms, city farms, schools, sanctuaries, institutions for disabled people, and private settings (Mattiello et al., 2024). In all the above-mentioned contexts, people are seldom fully aware of goats' behavioural and welfare requirements, and a better knowledge and understanding of the species, including the correct interpretation of goats' vocalisations, is of outmost importance to ensure goats' welfare and effective human-goat interactions (Celozzi et al. 2022).

Recent studies have provided knowledge on the influence of emotions on behaviour, vocal production, physiological parameters and cognitive abilities of goats (Siebert et al. 2011; Briefer et al. 2015; Baciadonna et al. 2016). In terms of vocal production, there is evidence that goat vocalisations vary with changes in emotional valence and arousal. In high-arousal situations, goats produce more vocalisations characterised by higher fundamental frequency (*fo*) and fundamental frequency contour parameters (*fo*mean and *fo*end), a distribution of energy concentrated on higher frequencies (Q25, Q50 and Q75), as well as a reduction in frequency range and entropy (Siebert et al., 2011). In positive situations, goat bleats show more consistent fundamental frequencies than those emitted in negative contexts ((Briefer et al. 2015); for the definition of vocal parameters, see (Briefer and McElligott 2012)).

To our knowledge, only one study has assessed human's ability to interpret emotional valence and arousal in goat bleats so far (Greenall et al. 2022). In this study, participants were asked to assess the emotional content, in terms of arousal or valence, of pairs of vocalisations produced in situations of known emotional arousal and valence by domestic (cattle, pigs, horses, goats) and wild ungulates (wild boar, Przewalski's horse). It emerged that participants rated arousal and valence of goat vocalisations above chance level, with a correct recognition percentage of 57% and 56% respectively. Goats ranked third in arousal recognition after pigs and horses, and fifth in valence recognition after humans, horses, wild boars and pigs. In particular, the arousal of goat vocalisations was better recognised than that of human speech (correct recognition rates of 57% and 55%, respectively). Interestingly, several factors such as age, familiarity, empathy, and specific features of the calls affected these scores.

The present study aims at improving our knowledge on humans' ability to understand goats' vocalisations and to identify their underlying emotions, depending on previous experience with goats, level of empathy towards goats, and other individual characteristics that may affect animals' emotion recognition.

2. Materials and methods

2.1. Participants

Participants had to be at least 18 years old and were recruited on a voluntary basis, by word of mouth, by posting announcements on the Facebook pages and websites of research projects on goats (e.g., the VOCAPRA project on the study of goat vocalisations - https://vocapra.lim.di.unimi.it/) and of organisations involved in goat farming and breeding (e.g., farmers' organisations), and by distributing leaflets with a QR code leading to the web page with the questionnaires during public events (e.g., conferences and meetings).

Participation was voluntary, anonymous and not remunerated. Prior to starting the survey, potential participants had to sign an informed consent. They were told that the purpose of the study was to investigate humans' understanding of goats' vocalizations and that they would have to fill in an online questionnaire and to listen to some goat vocalizations.

2.2. Goats' vocalizations

Spontaneous vocalisations of female Saanen and Alpine goats from four farms were recorded for one year using environmental sensors (SensorTile sensors from STMicroelectronics) in the frame of the VOCAPRA project (for further details on vocalisation recordings, please see (Ntalampiras et al. 2023). From this wide pool of vocalisations, we selected those emitted in four contexts that could potentially induce different emotional states. The selected contexts were:

- Pre-partum labour (PPL-): during the contractions that precede the expulsive phase of parturition.
- Mother-kid separation (MKS-): when the kid is separated from the mother 12 hours after birth (i.e., after the creation of a mother-infant bond) and allocated into a different pen with other kids, yet maintaining a constant visual, acoustic, tactile and olfactory contact with the mother through neighbouring pens.
- Mother-kid reunion (MKR+): when the offspring are reunited with their dams for suckling. This situation occurred only in one of the farms involved in the study, where kids were allowed to enter the pen of their dams three times per day from birth to one month of age and then twice a day until weaning, which occurred at 60-70 days of age.
- Waiting for feed (WFF+): when the farmer or the stockperson was administering hay, concentrate or unifeed in the feed through.

Both pre-partum labour and mother-kid separation are expected to trigger negative emotions (Olsson et al., 2004; Addae et al., 2000, respectively), while further studies are needed to explore the motivation of the mothers to reunite with their kids for suckling. However, since in nature the kids stay with their mothers until they are 11-12 months old, it can be inferred that, if mothers are given sufficient time to bond with their offspring, mother-infant contact after separation would evoke positive emotions in the dams (Fabre-Nys 1999). Based on previous studies, waiting for feed is also supposed to be a situation that triggers positive emotions in goats (Baciadonna et al. 2019).

From each of the four selected contexts, the mediods (the most representative sounds of each context) were extracted and saved as WAV files. Since the recordings were composed of several consecutive bleats of the same class, only one bleat was isolated for each mediod, whose vocal parameters are shown in table 1. The oscillograms, mean spectrum and spectrograms of the isolated bleats are reported in the appendix of the article. The vocal parameters seem to confirm our *a priori* hypothesis about the emotional valence of the isolated bleats related to each emission context, with shorter calls emitted in the two positive (mother-kid reunion and waiting for feed) than in the two negative (pre-partum labour and mother-kid separation) contexts (Briefer, 2020).

Table 1. Vocal parameters of the most representative sounds (mediods) of each context and their description, based on (Briefer and McElligott 2012). Contexts: PPL-: pre-partum labour; MKS-: mother-kid separation; MKR+: mother-kid reunion; WFF+: waiting for feed

			Contexts			
Vocal parameter	**Description**		**PPL-**	**MKS-**	**MKR+**	**WFF+**
Duration (s)	Total duration of the vocalisation		1.19	1.62	0.82	0.91
Energy quartiles (Hz)	Amount of energy in various part of the spectrum, ratio between harmonics/f_o. It is related to the distribution of energy in the spectrum	Q25%	257.4	609.6	613.0	874.0
		Q50%	579.8	1008.4	1282.2	1802.4
		Q75%	1197.0	2358.4	2228.7	2530.9
Fundamental frequency 'f_o' (Hz)			97.8	301.5	259.3	326.5
Peak frequency (Hz)	Frequency value with the maximum amplitude		187.5	625.0	531.3	687.5
Amplitude modulation or '*AM*' (dB)	Variation in amplitude relative to the total duration		18.4	17.6	12.8	13.7
Frequency modulation or '*FM*' (Hz)	Dominant frequency or f_o variability/modulation across the call		6.39	33.4	96.7	15.0
Harmonicity	Also called 'harmonic-to-noise ratio'; ratio of amplitude peaks of detectable harmonics to noise threshold (higher values indicate more tonal vocalisations)		-0.20	3.28	-0.45	3.14
Wiener entropy (logarithmic scale)	Ratio of the geometric mean to the arithmetic mean of the spectrum (0: pure tone; 1: random noise		-0.47	-1.27	-0.97	-0.93

2.3. Questionnaire

The whole questionnaire was built using online Microsoft Forms and included three sections.

The first section included a privacy statement (see Ethical statement) and provided general information on the project and on its aims, informing the participants about the potential spin-offs of the study, i.e. how the results could contribute to improving the quality of the human-animal relationship, with a positive impact on goats' welfare. This section also included some questions related to demographic variables (e.g., gender, age, profession, level of education) and to background experiences with animals and in particular with goats).

The second section explored some aspects of empathy toward goats in participants without frequent/direct contact with goats and with frequent/direct contact with goats (i.e. farmers, technicians and veterinarians). In particular, from the section devised at investigating factors affecting humans' empathy and attitude towards animals, we selected four items focusing specifically on goats: 1) I will almost always get in a good mood when I see healthy and happy goats; 2) It is silly to become attached to a goat; 3) It amazes me how upset some farmers become when they have to kill a goat; 4) It upsets me to see and hear about goats that have been killed by predators. Respondents were asked to express their agreement on a Likert scale ranging from 1 (total disagreement) to 7 (total agreement). Two items (statements 2 and 3) were turned negative to avoid response bias, and the answers to these questions were therefore reversed before being analysed. Responses to each item were summed for each respondent, and the final total scores were divided into three score bands corresponding to the 25th (<18; low empathy), 50th (<21; medium empathy) and 75th percentile (>21; high empathy).

Finally, in the third section participants were asked to listen to four bleats (emitted in the four above-mentioned contexts, i.e., pre-partum labour, mother-kid separation, mother-kid reunion, waiting for feed,) and to describe the valence and arousal of goats' emotions expressed by each bleat, by attributing a score on a seven-point Likert scale to eight descriptors: Aggressive, Active, Curious, Frustrated, Irritated, Relaxed, Satisfied, Suffering–in pain. Higher scores described a higher perceived intensity of the descriptor. These descriptors were selected from the existing fixed list of terms developed in the AWIN project for the Qualitative Behaviour Assessment of goats (AWIN 2015). Participants were then asked to associate each bleat with one of four emission contexts. Before starting the third section, participants were provided with specific instructions on how to listen to the bleats, i.e. in a silent environment, possibly with earphones or, even better, with closed headphones. If they were using a mobile phone, they were advised to hold it in a horizontal position to display all the bleating descriptors correctly. They were also informed that they could listen to the bleats as many times as they wanted, before scoring them and assigning them to one of the four contexts.

The questionnaires were made available online on the VOCAPRA website (https://vocapra.lim.di.unimi.it/survey.php) in December 2022. In the present manuscript, we are presenting data collected until January 2024.

Statistical analysis

For each context of emission, a Chi-square test was used to compare the frequencies of correct attribution of the vocalisation to that context with the frequencies expected by chance (0.25) and a Principal Component Analysis (PCA; Proc PRINCOMP; SAS, 2012) was performed on the scores of qualitative descriptors that were attributed by the respondents to each vocalization, to understand how respondents perceived the emotional content of bleats emitted in different contexts of emission.

Furthermore, for each context a logistic regression analysis (Proc LOGISTIC; SAS, 2012) was performed to investigate the effect of demographic factors and the level of empathy on the human ability to discriminate goats' vocalizations depending on the context of emission, and recognition rates for each factor were compared by Chi-square test. The following factors (and correspondent levels) were taken into account: gender (male *vs* female), age class (18-29, 30-49, ≥50 years old), marital status (partner - which includes partner, married - *vs* no partner - which includes divorced, unmarried and widow/widowed -), whether the participants had children (yes or no), University education (i.e. if the participants have a University degree; yes or no), type of university education (scientific or humanistic subject), grown up with pets (yes or no), and if they were currently living with pets (yes or no). Furthermore, the respondent's empathy level (low, medium and high) was also included in the model. Logistic regression analyses examined the effect of these factors and of all their possible interactions on the correct/incorrect classification of the context of emission and excluded variables and their interactions by means of a stepwise backward multiple regression method, with an input and output significance of 0.2. The end results of the logistic analyses were final models including all variables significantly associated with human bleating recognition skills. The models were described in terms of odds ratios, with 95% confidence intervals.

3. Results and discussion

Three hundred and thirty-one participants took part to the survey, but only 290 questionnaires were completely filled and were therefore retained for further analysis. The participants to these 290 questionnaires were adults, aged between 18 and 74 years (33.8±12.9 years); 193 were females and 97 were males. Two hundred and seventeen participants had no frequent direct contacts with goats, while 73 were farmers, technicians, or veterinarians with frequent contacts with goats. Respondents were able to correctly discriminate the bleats' context of emission significantly above the chance level (25%) for three out of four emission contexts (table 2). Vocalisations associated with contexts in which the underlying emotions were positive (MKR+ and WFF+) were less likely to be recognised by respondents than vocalisations related to situations eliciting negative emotions (PPL- and MKS-). In particular, the context referred to MKR+ was the least recognised by respondents and the level of its successful classification was below the chance level. However, when summing the correct answers of the two positive contexts, we observe that the respondents were able to correctly identify the positive valence significantly above the chance level (64.49% of assignment to a positive context for MKR+ and 85.52% for WFF+; $p<0.001$ in both cases). Our research confirms that the two negative contexts are correctly assessed both for the emotional valence and for the specific context in which the bleat was emitted. This is possibly due to the fact that, in spite of some exceptions (e.g., chickens; (McGrath et al. 2024)), acoustic signals emitted in negative contexts are likely to convey information about possible dangers and are therefore more important in interspecific communication, as suggested by research on piglets, cats, dogs, and chimpanzees (Tallet et al. 2010; Scheumann et al. 2014; Prato-Previde et al. 2020). This is in line with the results from studies in pigs and dogs, where the vocalisations most recognised by the respondents were those recorded during negative events for the animals, such as castration in pigs (Tallet et al. 2010) and when dogs were alone or in presence of strangers (Pongrácz et al. 2005).

An additional explanation for the higher recognition rate in negative contexts lies in the fact that the two negative contexts differed in the level of arousal, as confirmed by some vocal parameters. In fact, bleats emitted during PPL- were characterised by a distribution of energy concentrated on lower frequencies, a lower fundamental frequency (*fo*) and a higher entropy (Table 1), which are all suggested as markers of low arousal in goats (Briefer et al., 2015; Siebert et al., 2011). On the contrary, MKS- and both MKR+ and WFF+ were characterised by a distribution of energy concentrated on higher frequencies, a higher fundamental frequency (*fo*) and a lower entropy, which indicate a condition of high arousal. Therefore, the difference in arousal could probably be of help in discriminating between the two negative contexts, whereas it was more difficult to distinguish between the two positive contexts, which have similar arousal levels.

Table 2. Correct assignment of bleats to each context of emission (absolute frequencies and percentages). Significance levels refer to the correct proportion of context assignments that are above chance level (25%). Contexts: PPL-: pre-partum labour; MKS-: mother-kid separation; MKR+: mother-kid reunion; WFF+: waiting for feed

	PPL-	**MKS-**	**MKR+**	**WFF+**
PPL-	224 (77.24%) ***	25 (8.62%)	27 (9.31%)	14 (4.83%)
MKS-	26 (8.97%)	172 (59.31%) ***	57 (19.7%)	35 (12.07%)
MKR+	44 (15.17%)	59 (20.30%)	72 (24.83%)	115 (39.66%)
WFF+	4 (1.38%)	38 (13.10%)	110 (37.93%)	138 (47.59%) ***

***: $p<0.001$

This is reflected in the results from PCA on the eight qualitative descriptors of goat vocalisations assigned by the respondents to each bleat: in Figure 1, a clear separation trend between positive and negative contexts can be observed, with positively-valenced contexts (MKR+ and WFF+) clustering on the right side of PC1, characterised by high values of the four positive descriptors (active, curious, relaxed and satisfied), and negatively-valenced contexts (PPL- and MKS-) on the left side, characterised by high values of descriptors of negative emotional state (irritated, frustrated, aggressive and suffering).

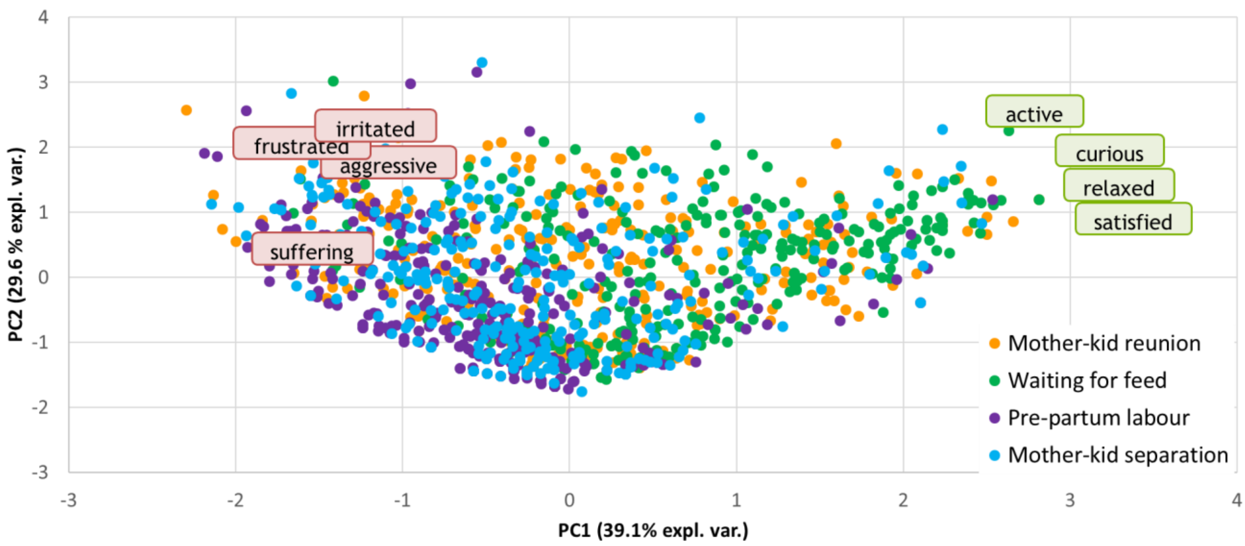

Figure 1. Distribution of goat vocalisations on PC1 and PC2 depending on the scores of the eight qualitative descriptors

In terms of emotional valence, the results of the present study confirm the human ability to discriminate between positive and negative emotions conveyed by vocalisations, as observed in goats (Greenall et al. 2022), as well as in various animal species such as cats, domestic horses, pigs and wild boars (Tallet et al. 2010; Prato-Previde et al. 2020; Greenall et al. 2022). (Greenall et al. 2022) observed how certain vocal parameters of the vocalisations of various domestic and wild ungulates, including goats, influenced their emotional perception by humans: goat bleats were perceived as more positive when characterised by a high spectral centroid and lower amplitude modulation (*AM*). This is in line with results of the present study, being positive calls characterised by a lower *AM* than negative sounds (Table 1).

Regarding the discrimination of arousal level, Figure 1 shows that the bleats were not distributed according to this emotional dimension, indicating that this was not perceived by the participants. This finding is apparently in contrast with the existing literature, which suggests that emotional arousal is more likely to be detected than valence in the cross-species recognition of emotion through acoustic cues, based on the hypothesis that vocal markers of emotional arousal would have been conserved throughout the evolution process, unlike those of valence, which might be more species-specific (Filippi et al. 2017; Greenall et al. 2022). However, as previously mentioned, in our study the level of arousal was similar in three contexts (MKS-, MKR+ and WFF+), characterised by a distribution of energy concentrated on higher frequencies, higher fundamental frequency (f_o) and a lower entropy; Table 1) and differed only for the PPL- bleat, which was the only low-arousal context, and this may justify the scarce difference in its perception by the respondents (Figure 1).

Although usually above the chance level, participants' recognition rates of emotion-generating contexts of bleats were not very high, despite goats' long history of domestication (started 10,000 years ago) was supposed to favour an effective recognition by humans of emotional expression of their vocalisations. In fact, (Greenall et al. 2022) reported a higher recognition rate of emotional arousal from vocalisations produced by domestic ungulates, including goats, rather than by wild ones. On the other hand, relatively low percentages of correct assignment of emission contexts have been observed even in some companion animals, that live in close contact with humans. For example, (Prato-Previde et al. 2020) found that recognition rates of cats' meows by humans ranged from 26.7% for meows emitted during isolation (negative context) to 40.4% for those emitted while waiting for food (positive context).

Furthermore, it should be noted that interspecific communication between humans and goats is based also on visual and tactile signals (Celozzi et al. 2022), and therefore the lack of visual support (behaviours and facial expressions) and of direct contact make it more difficult for humans to classify animal vocalisations, as suggested for cats by (Nicastro and Owren 2003). Furthermore, the fact that most of the respondents had no frequent contact with goats reduced the rate of correct answers, as shown by the results of the logistic regression analysis. This analysis allowed the identification of the most important factors that, in combination with the other factors, affect the human ability to assign goat vocalisations to each correct context of emission. In all the four contexts, experience with goats significantly entered the models (Table 3), highlighting the importance of this factor on the ability to discriminate vocalisations emitted by goats: people with frequent direct contact with goats were more likely to assign bleats to the correct context of emission than people who were unfamiliar with these animals.

Table 3. Logistic regression analysis (stepwise backward multiple regression method, with an input and output significance of 0.2) of demographic factors and empathy level associated with each context

Effect	Odds ratio	95% Confidence interval	p
		pre-partum labour	
Experience with goats (yes *vs* no)	2.28	1.05-4.97	0.010
Children (yes *vs* no)	0.31	0.16-0.59	0.002
Currently living with pets (yes *vs* no)	1.67	0.92-3.05	0.092
Empathy with goats (low *vs* high)	0.44	0.21-0.90	0.027
Empathy with goats (medium *vs* high)	0.80	0.38-1.68	0.027
		mother-kid separation	
Experience with goats (yes *vs* no)	2.61	1.44-4.74	0.001
		mother-kid reunion	
Experience with goats (yes *vs* no)	1.577	0.88-2.84	0.127
		waiting for feed	
Experience with goats (yes *vs* no)	1.64	0.95-2.83	0.049
Female *vs* male	0.60	0.36-0.99	0.048
University education (yes *vs* no)	1.68	1.02.-2.76	0.072
Currently living with pets (yes *vs* no)	1.49	0.88-2.53	0.137

These results were confirmed also at univariate level by Chi-square test, although experience with goats alone was not always able to highlight significant differences. The percentages of correct classification of bleats were 84.9% *vs* 74.7% for PPL- (Chisq=3.28; n.s.), 75.3% *vs* 53.9% for MKS- (Chisq=10.39=10.4; p<0.01), 31.5% *vs* 22.6% for MKR+ (Chisq= 2.33; n.s.) and 57.5% *vs* 44.2% for WFF+ (Chisq=3.87; p<0.05) for people with and without experience with goats, respectively.

This finding is in line with some previous studies showing that the human ability to correctly interpret the emotional state of another species from vocalizations is affected by familiarity and experience with it (Scheumann et al. 2014; Faragó et al. 2017; Prato-Previde et al. 2020; Greenall et al. 2022). However, when assessing humans' ability to correctly interpret vocalizations of domesticated ungulates, (Greenall et al. 2022) reported only tenuous evidence that participants' working with animals performed better in interpreting ungulates' vocalizations than participants not working with animals.

Additional factors were retained in the final models for PPL- and for WFF+. For PPL-, the level of empathy with goats significantly entered the model (low *vs* high and medium *vs* high). Interestingly, even though empathy significantly entered only this model and none of these differences reached statistical significance at univariate level (Chi-square test), the trend of correct answers depending on the level of empathy was identical for all the four contexts: people with a low or a medium level of empathy had a significantly lower probability to discriminate this bleat than people with a high empathy level (Figure 2).

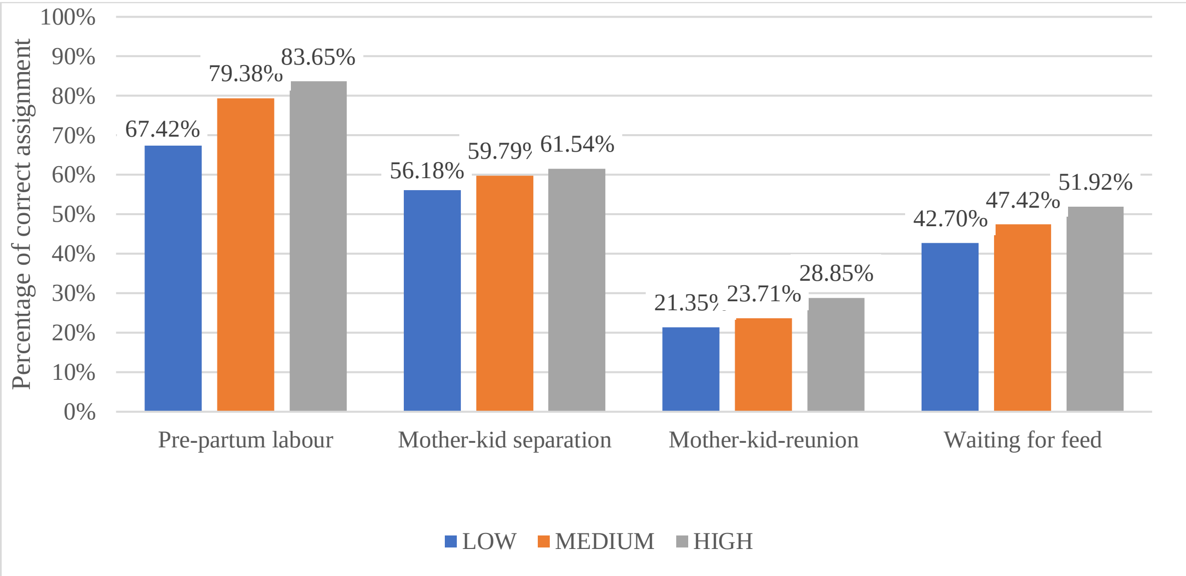

Figure 2. Percentage of correct assignment in the four contexts, depending on the level of empathy

Besides previous experience with a species, empathy has been reported as a potential influencing factor of human's ability to interpret affective states in other species (Kujala et al. 2017; Prato-Previde et al. 2020; Greenall et al. 2022; Prato-Previde et al. 2022). People scoring higher on empathy perform better in interpreting emotions (dogs: (Kujala et al. 2017); cats: (Prato-Previde et al. 2020); different ungulate species, including goats: (Greenall et al. 2022)) compared to people scoring lower on empathy. This can be explained by the fact that both cognitive and affective dimensions of empathy are associated not only with a better emotion recognition ability but also to more positive human animal relationships, animal caring and animal welfare (Ellingsen et al. 2010; Norring, Wikman, A.H. Hokkanen, et al. 2014; Prato-Previde et al. 2022). In the current study empathy towards goats was assessed using a simplified scale based on four statements, thus a more in-depth analysis will be carried in the future using the complete questionnaire. This will hopefully help to strengthen the results and to better understand the role of empathy on the interpretation of goats' emotions.

Another factor that significantly entered the model of the PPL- context was living with pets: people living with pets had a higher recognition rate (81.5% *vs* 67.8% for people living with pets *vs* people who do not currently live with pets, respectively; Chisq=6.65; $p<0.05$). This factor significantly entered the model also for WFF+ (50.0% *vs* 42.2% for people living with pets *vs* people who do not currently live with pets, respectively), although univariate differences were not statistically significant (Chisq=1.51; n.s.). Also in this case, even though differences were not always significant, the trend of recognition rate was the same in all the four contexts (Figure 3). There is evidence that living with pets (pet ownership) may affect accuracy in identifying emotional states in companion animals such as dogs and cats ((Amici et al. 2019; Hawkins et al. 2021 Dec; Prato-Previde et al. 2022)). Living with pets entails having daily direct experience with another species and may promote general beliefs about animal emotional life also fostering empathy towards animals (Kujala et al. 2017; Hawkins et al. 2021 Dec; Braun et al. 2024).

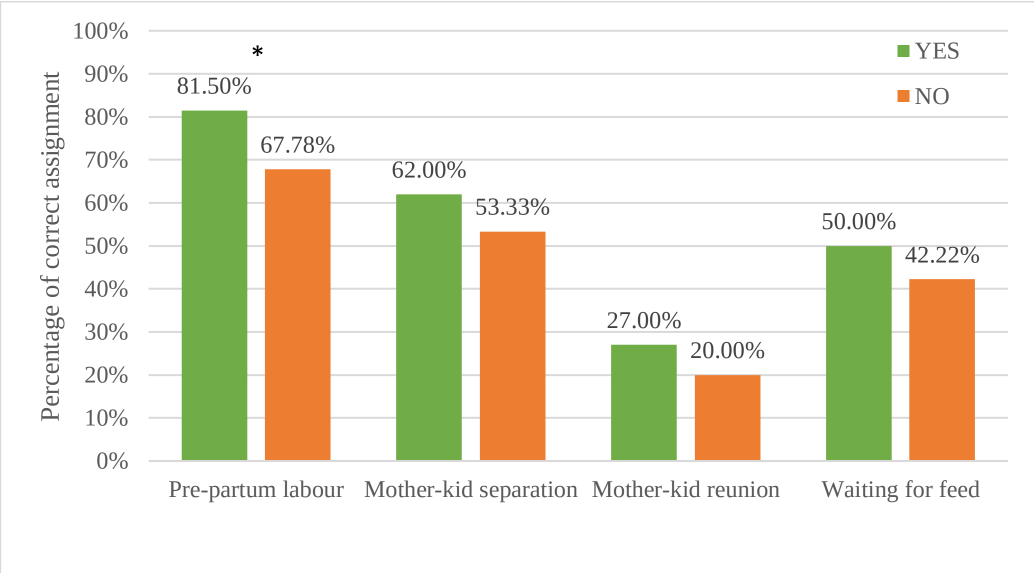

*Figure 3. Percentage of correct assignment in the four contexts, depending on the factor "living with pets" (Significance: * p<0.05)*

The presence of children significantly entered the model for PPL-, with people not having children scoring higher than people with children (81.5% *vs* 63.2% for people who do not have children *vs* people with children, respectively; Chisq=9.91; $p<0.05$). However, it is worth noting that PPL- was the only context where these differences were significant: in all the other contexts no significant differences emerged and the trend was reversed, with people with children showing a higher recognition rate for MKS- (61.76% *vs* 58.56%), MKR+ (26.47% *vs* 24.32%) and WFF+ (55.88% *vs* 45.05%). These inconsistent results are difficult to explain. Furthermore, the only significant result is opposite to expectations. Since empathy probably evolved from the mother–offspring bond (Preston and de Waal 2002), people with children - and particularly women - could be better at recognizing the emotions of others. However, (Greenall et al. 2022) found no effect of having children on participants' correct ratings of vocalizations and concluded that the ability to judge animal emotions seemingly does not depend on these demographic factors.

In our study the gender of the respondents significantly entered the model for WFF+, with males showing a higher recognition rate than females (55.67% *vs* 43.52%, respectively; Chisq=3.82; p=0.051). Males performed better than females also in the MKS- context (61.86% *vs* 58.03%, respectively; Chisq=0.39; n.s.), whereas females scored better than males in the PPL- (81.35% *vs* 69.07%, respectively; Chisq=5.53; p<0.05) and in the MKR+ (25.91% *vs* 22.68%, respectively; Chisq=0.36; n.s.) contexts. These results are not consistent across contexts and only partially in line with previous literature on gender differences in animals' emotion recognition. Studies on intraspecific recognition of nonverbal communication signals (e.g., vocal affect, facial expressions, body language) suggest a significant main effect of gender (Scherer and Scherer, 2011) with females in general outperforming males (Collignon et al. 2010). On the other hand, studies on interspecific emotion recognition suggest a potential gender bias in recognizing the emotional states of animals with women being more accurate than men ((Nicastro and Owren 2003; Tallet et al. 2010; Schirmer et al. 2013; Faragó et al. 2017; Dawson et al. 2019; Prato-Previde et al. 2022). For example, a recent study (Dawson et al. 2019) showed that people can identify cats' emotions from their faces and that women are more successful than men in the identification of cats' emotions; similarly, (Schirmer et al. 2013) reported that women are more sensitive than men to dogs' affective expressions. Regarding vocal behaviour, a limited gender effect that approached significance was observed on the ability to recognize cats' (Nicastro and Owren 2003) and piglets' (Tallet et al. 2010) vocalizations. More recently, (Prato-Previde et al. 2022) found that females were significantly more accurate than men in interpreting cats' meows during isolation and brushing, whereas no gender differences were found by (Greenall et al. 2022) for the interpretation of animals' emotions in different species of domesticated or wild ungulates.

Finally, we found that bleats emitted in the WFF+ context were more likely recognised by people with a university degree (52.0% of correct assignment) than by people without a degree (40.7% of correct assignment), although Chi-square test highlighted that this difference only approached statistical significance (Chisq=3.51; p=0.06), while in the other contexts negligible and non-significant differences were found in response to the level of education. Educational level was not found to be associated with the correct assignment of calls to emission contexts in piglets or with the human ability to decipher the emotions conveyed by the vocalisations of domestic and wild ungulates (Tallet et al. 2010; Greenall et al. 2022). The limited effect of this factor seems to be confirmed by our results; however, further studies are needed to better understand its role in understanding animals' emotions.

4. Conclusions

Our results highlighted a good humans' ability to identify the valence of emotions expressed by goats' vocalizations, as shown by the attributes used by the respondents to describe the vocalisations, which were coherent with the context of emission. Furthermore, the respondents were able to correctly discriminate the bleats significantly above the chance level for three out of four emission contexts, even though this capacity was higher for negative contexts, probably due to the greater importance of the information conveyed by the calls emitted in negative than in positive situations.

Experience with goats was consistently related to a higher correct classification rate, suggesting that, to ensure good welfare levels, the presence of experienced people is important not only in goat farming, but also in all the other contexts where interactions between the two parties may occur.

A high level of empathy with goats also seems to improve the recognition of emission contexts, although the role of empathy and attitudes and their underlying factors deserve further investigation. Other individual characteristics such as having children, gender and university education showed inconsistent results and therefore require further research.

5. Ethical statement

The questionnaires were approved by the Ethics Committee of the University of Milan. A privacy statement was included in the general information section of the questionnaires, in which the processing of respondents' personal data was made explicit in accordance with Italian law, art. 13 of Reg. 2016/679 (GDPR). Furthermore, it was stated that participation in the questionnaires was free of charge and that the answers would be collected anonymously. The approval of the Animal Welfare Organisation of the University of Milan was not required for this study, as no animal manipulation or modification of the normal husbandry routine took place during data collection, and the goats were maintained and observed in their usual housing systems, under their usual husbandry routine, throughout the study period.

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7. Appendix

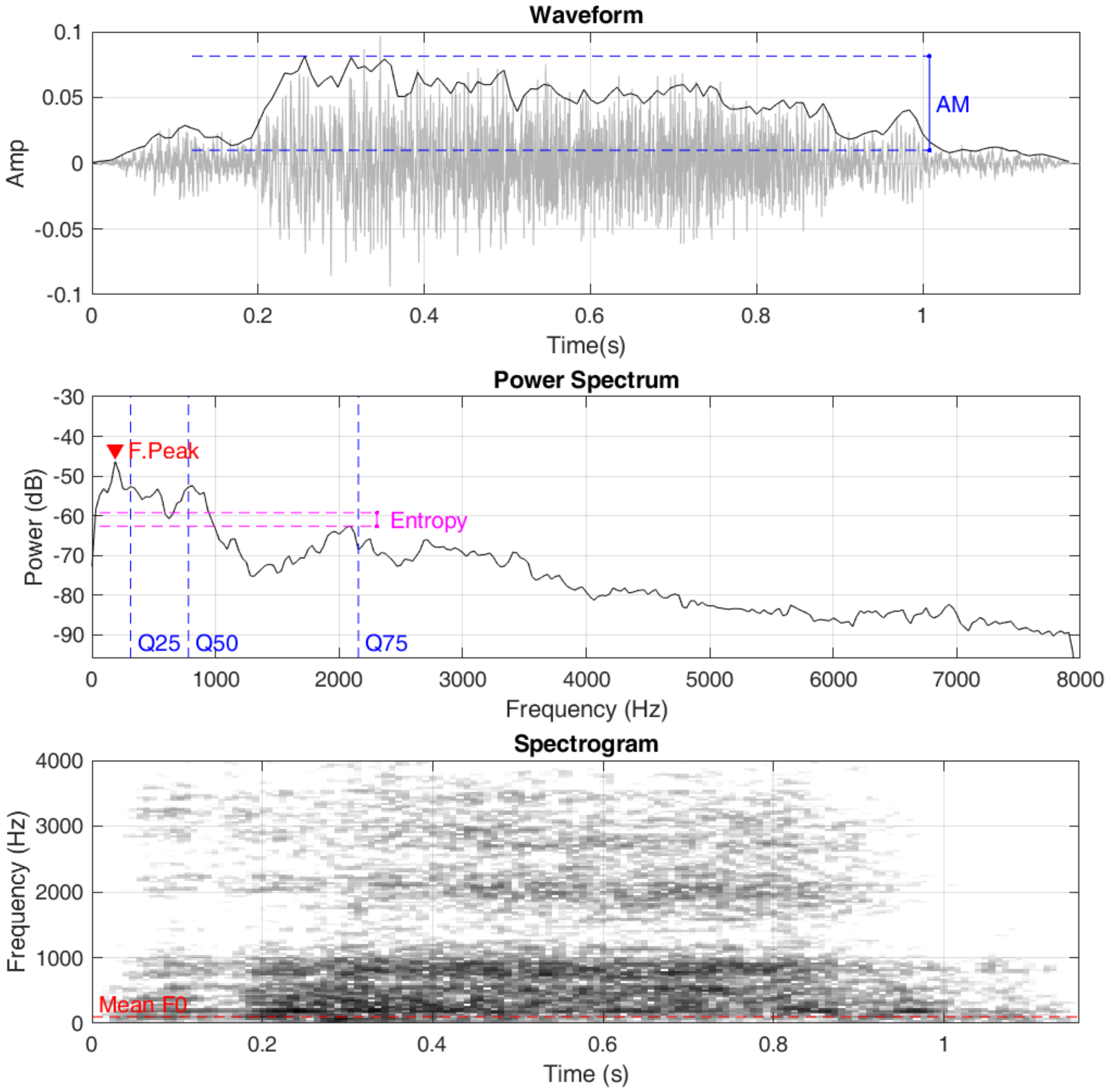

Figure A1. From top to bottom: oscillogram, meanspectrum and spectrogram of the bleat isolated from the mediod of pre-partum labour

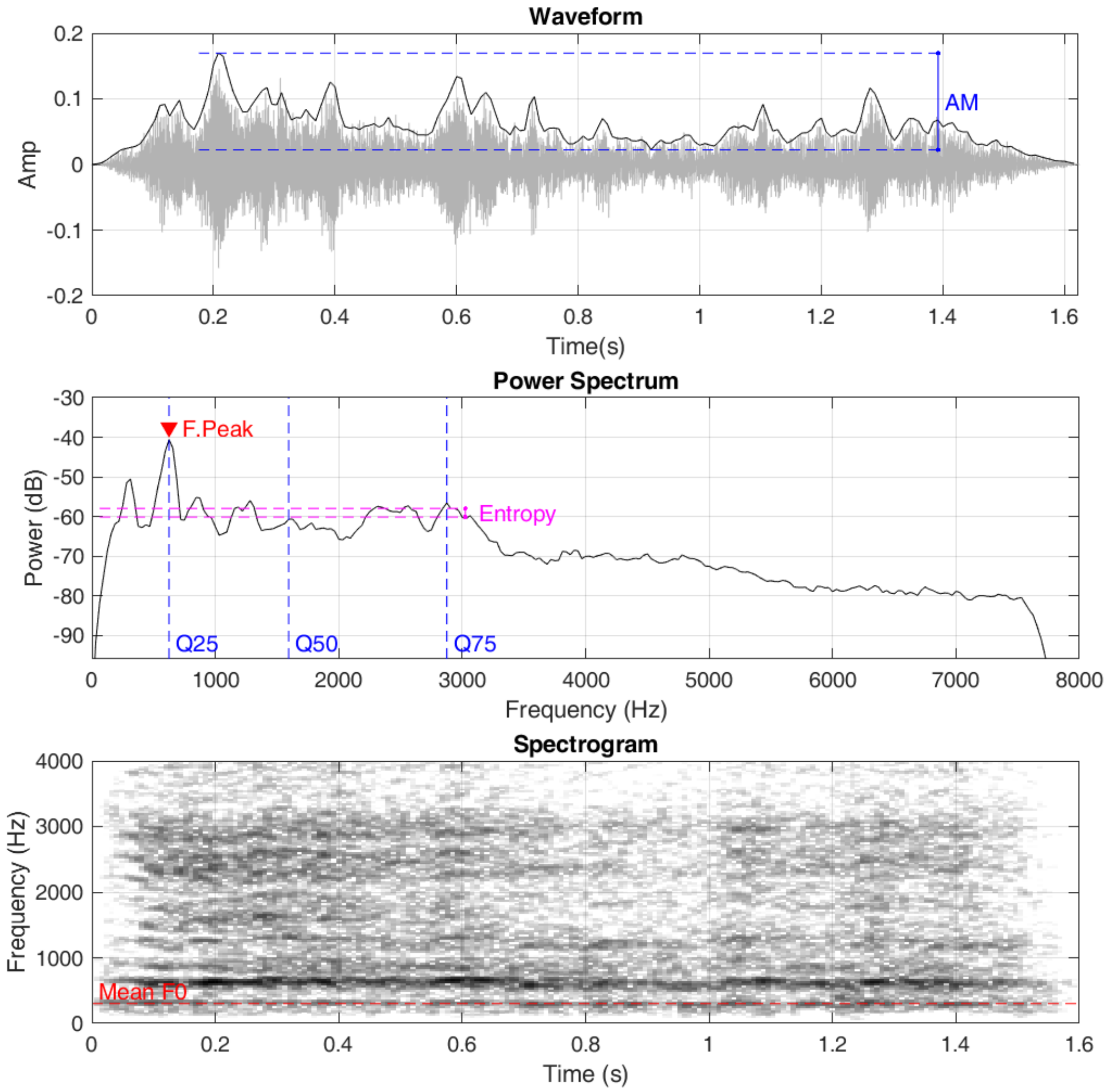

Figure A2. From top to bottom: oscillogram, meanspectrum and spectrogram of the bleat isolated from the mediod of mother-kid separation

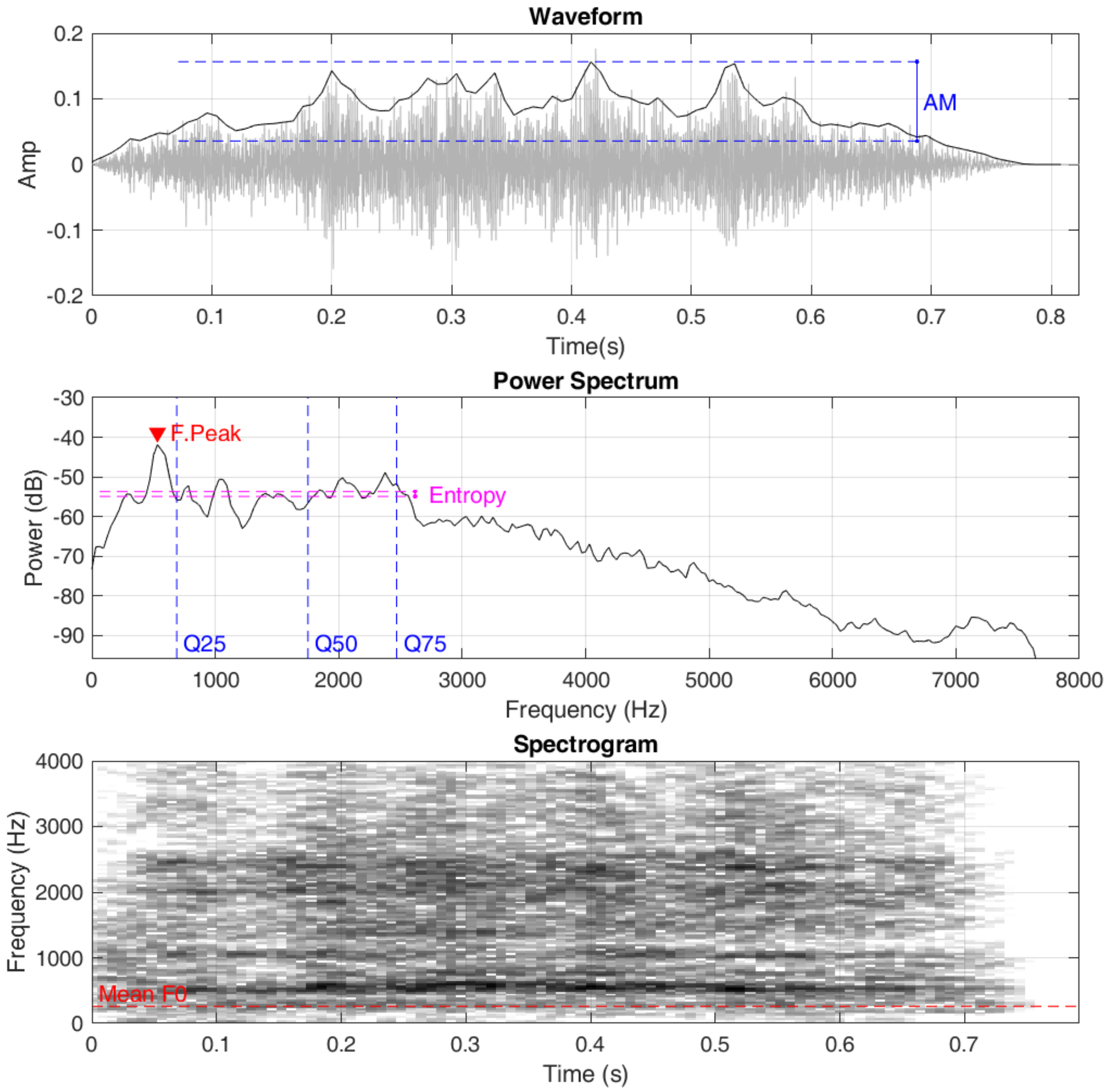

Figure A3. From top to bottom: oscillogram, meanspectrum and spectrogram of the bleat isolated from the mediod of mother-kid reunion

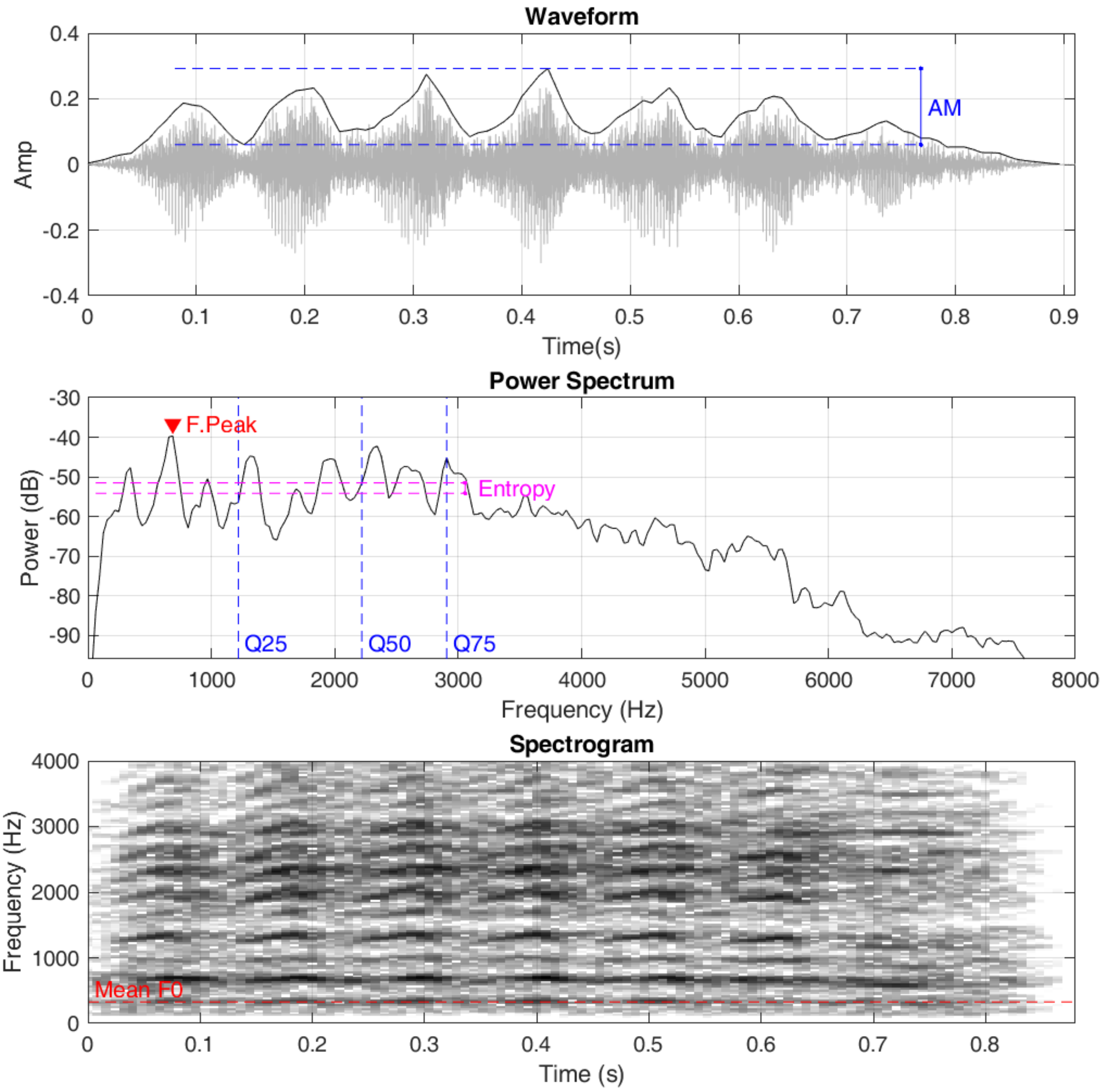

Figure A4. From top to bottom: oscillogram, meanspectrum and spectrogram of the bleat isolated from the mediod of waiting for feed

5.3 ACOUSTIC MARKERS OF NEGATIVE AROUSAL IN LAMBS (*OVIS ARIES*): EVIDENCE FROM BEHAVIOURAL AND EYE THERMAL PROFILES

Celozzi, S., Briefer, E. F., Miot, Z., Renaud-Goud, P. N., Mattiello, S., Villain, A. S. M. (2024)

Ready for submission to: *Animal Behaviour*

1. Introduction

Emotions can be defined as short-term multi-component responses resulting from rewarding or punishing stimuli or events (Mendl and Paul, 2020). Among the theories of emotions, the dimensional theory is one of the most widely used in non-human animals (hereafter 'animals'). According to this theory, emotions are characterised by two dimensions: valence, which can be positive (pleasant) or negative (unpleasant), and arousal (bodily activation), which can be low or high (Russell 1980; Mendl et al, 2010). Emotions trigger neuro-physiological, behavioural and cognitive changes that can be quantified in animals, allowing us to assess the biological aspects of emotional states, but not the subjective feeling that is accessible so far only in human species.

The adoption of the dimensional theory has allowed the identification of several indicators of emotional valence and arousal in animals, including behavioural (e.g., ears and tail postures in sheep, *Ovis aries*, Reefmann et al., 2009a; body postures in cows, *Bos taurus*, de Oliveira and Kneeling, 2018; head movements, moving, ears and tail postures and vocalizations in goats, *Capra hircus*, Briefer et al., 2015a; tail and ears movements in pigs *Sus scrofa*, Rius et al., 2019), physiological (e.g., cardiorespiration and body surface humidity in sheep, Reefman et al., 2009b, nasal temperatures in dairy cows, Proctor et al., 2015, heart rate in laying hens, Davies et al., 2014), and cognitive indicators (e.g.,; judgements bias in goats, Baciadonna et al., 2016).

Infrared thermography is a technique for indirectly assessing the temperature of a subject by measuring the intensity of infrared radiation emitted by it. Recently, thermography has been used to study emotions in animals, however with a strong focus on negative emotions such as stress and pain (Travain and Valsecchi, 2021). Temperature is not an indicator of arousal that can be generalised to all areas of an animal's face and body, as they exhibit different thermal behaviours. Indeed, increasing the level of arousal leads to a decrease in nasal temperature in marmosets (Ermatinger et al., 2019), a decrease in tail and paw temperature in rats (Vianna and Carrive, 2005), and an increase in eye temperature in dogs (Rigterink et al., 2018). In sheep, thermography has been used to assess the animals' response to negative emotions such as pain and fear, and in response to stress. However, these studies did not provide clear information on the level of emotional arousal (Comin et al., 2023; Stubsjøen et al., 2009), or considered only one combination of valence and arousal, such as a situation of negative valence with a high intensity (i.e., shearing; Arfuso et al., 2022). Exposure of sheep to stressors such as tethering (Cannas et al., 2018), shearing (Arfuso et al., 2024) and fear (opening of an umbrella; Comin et al., 2023) leads to an increase in eye temperature in sheep. The stress of transport causes an increase in core temperature in these animals (Ingram et al., 2002), while the stress of social isolation causes a decrease in ear pinna temperature due to vasoconstriction (Lowe et al., 2005). From the evidence available so far, it is clear that more research is currently needed to determine the thermal behaviour of different parts of the body, including the eye, in situations characterised by similar valence but different emotional arousal in the sheep species.

It has recently been shown that vocalisations can be used as a non-invasive indicator of animal emotion. Several scientific studies have identified vocal correlates of arousal and valence, albeit with a bias towards the former. Some vocal features change similarly with varying arousal across species, while vocal correlates of valence might be more species specific (Briefer, 2020). In high arousal contexts, calls are often louder, have higher frequencies and a more variable fundamental frequency. In positive situations, animals tend to produce shorter vocalisations, with a lower and less variable fundamental frequency (Briefer, 2020). Thus, the existence of common rules linking variation in vocal characteristics with emotional valence and intensity across species makes vocalisations a candidate as a universal indicator of emotion (Briefer, 2020).

In sheep, vocalisations serve several functions, such as reinforcing maternal bonding (Sèbe et al., 2007) and mutual recognition (Papadaki et al., 2021), but are also emitted for the purpose of group cohesion (contact calls) and to signal stress (Dwyer, 2008). Sheep produce two types of bleats, low-pitched bleats (emitted with the mouth closed) and high-pitched bleats (emitted with the mouth open) as well as mixed bleats (starting with a closed mouth and finishing with opened mouth). Low-pitched bleats or “rumble” sounds are emitted by the mother in presence of her lamb, but also by sheep in response to fear (Dwyer, 2008). High-pitched bleats are produced in high-arousal positive contexts, such as waiting for or receiving food, but also in some high-arousal negative contexts, such as isolation from conspecifics and separation of lambs from their mothers (Dwyer, 2008). Therefore, the production of open-mouthed bleats seems to occur in situations characterised by a high level of arousal (Papadaki et al., 2021). Nevertheless, there are contexts in which vocal production in sheep is inhibited, such as in the presence of a predator (Dwyer, 2008).

Overall, little is known about the vocal expression of emotions in sheep. For instance, sheep vocalisations have never been linked to other measures of arousal such as behaviour and thermography for validation. With regard to acoustic features, the few studies on this species show an increase in f_o contour features (Sèbe et al., 2012) and a lower energy distribution (i.e., energy quartiles; Sèbe et al., 2012; Engeldal et al., 2013), with increasing negative emotional arousal. In addition, using isolation tests in ewes, Papadaki et al. (2021) found an increase in fundamental frequency-related features (min and mean f_o and f_o disturbance; Papadaki et al., 2021), as well as an increase in frequency distribution when ewes had no lambs under their care (dry period) compared to when they had lambs under their care (suckling period). The authors hypothesised that the changes in the acoustic features of sheep bleats in the isolation tests carried out during the dry period compared to the suckling period were due to a higher level of arousal experienced by the sheep. However, with the exception of the Sèbe et al. (2012), the other studies did not control for the level of emotional arousal, and therefore the current scientific evidence on the vocal correlates of this emotional dimension in sheep requires further investigation.

This study aims to fill knowledge gaps on potential indicators of negative emotional arousal in sheep from a behavioural and physiological perspective. Sheep (*Ovis aries*) are a gregarious and prey species, and separation from conspecifics is therefore perceived as a stressful and fear-provoking event (Cockram et al., 1994). Furthermore, it is presumable that an individual's response to isolation may vary depending on its level of sociability.

In this study, lambs were involved in a two-phases isolation test (partial vs full) in order to assess the behavioural response of lambs to social isolation in regards to their biometric characteristics (lamb size, i.e., lamb height) and social behaviour toward the audience (interactions with one or more members of the group) during the partial isolation. In addition, the changes in vocal features of lamb bleats as a function of the intensity of the behavioural response to isolation was also investigated. Finally, the relationship between the thermal profile of the lambs' face and vocal features and behavioural response to isolation was also taken into account.

We expected a greater behavioural response to isolation in more sociable individuals, irrespective of animal size. We predicted an increase in the bleats' frequencies as the intensity of the behavioural response to isolation increased and a positive relationship between vocalisation frequencies, eye temperature and the intensity of the behavioural response.

2. Materials and methods

2.1. Animals and housing conditions

The study was carried out on a commercial meat sheep farm in North Zealand, Denmark, from April to May 2023. The flock was composed by one hundred and ten sheep (*Ovis aries*) belonging to different breeds (Frisian milksheep, Lacaune, Charolais, Zwartbles, Texel, Spelsau Lleyn Suffolk and Dorset). Of these sheep, 58 were lambs aged between four and five months old at the start of the experiment, and 52 were ewes up to eight years old. All the animals were housed in the same stable of 200m^2 on a deep-litter straw bedding and had access to pasture from 8 am to 4 pm, accompanied by the farmer and sheepdogs. The pasture was composed by two areas of 15,100m^2

and 36,200m^2. All animals were managed in the same way in terms of feeding, which was calibrated to the nutritional requirements of adult sheep at approximately 320 g of barley per animal divided in two daily administrations, at 8 and 3 pm. All sheep had ad libitum access to water.

The animals were naive to any experiment and were used for two subsequent experiments. In the other experiment, sheep were exposed to a playback experiment in the field, involving no previous manipulation. This playback experiment occurred after the tests presented in this study.

2.2. Experimental procedure

2.2.1 Individual identification and habituation

All sheep were marked using specific sheep spray (Agrihealth Quickdry Sheep Marker Spray) according to a unique and specific colour code applied to the back and flanks of each animal. For the need of the other experiment running at the same time, the flock was divided into two homogeneous parts in terms of numbers, keeping the lambs together with their mothers. The barn was divided in two egal part and each group was assigned a pasture field. Hence, a habituation phase of two days was adopted to let the animals familiarize themselves with the division of the flock, the location of pasture field they were assigned to and the position of the feeders in the fields. The sheep were also habituated to the presence of two experimenters, who walked for 10 min twice a day in the fields for two days, maintaining approximately thirty meters from the animals. The sheep were also habituated to the equipment: a hide (supplementary material, figure S1), which was installed in the barn to allow the experimenters to observe the sheep without disturbing them.

During the habituation, the sheep were taken out (8:30 am) and in (4 pm) of the barn to go out to pasture accompanied by the farmer and the sheep dogs, and received barley twice a day (at approximately 8:30 am and 3 pm) at pasture.

2.2.2 Isolation tests

Isolation tests started two days after the habituation phase. A total of twenty randomly selected lambs (n=11 females; n=7 males, n=2 unknown) were involved in the study. The tested lambs were isolated from conspecifics in the morning (08:21 (h:min) ± 00:12) before the flock was turned out to pasture. Once on the pasture, the animals received the morning administration of barley. For each isolation test, a randomly selected lamb was gently restraint by two experimenters, and placed in a metal pen in the stable where the animals were normally housed. The pen was shaped like an irregular polygon, approximately 1.43 m high and 2m^2 in area (supplementary material, figure S1). The isolation test was separated into two phases: 1) the lamb had visual, vocal and physical access through the grid of the pen with its group members (partial isolation); 2) the group was guided toward the pasture field by one experimenter, while the tested lamb remained in the isolation pen in the absence of physical, visual or vocal contact with group members (full isolation). Each phases lasted 4 minutes, for a total duration of 8 minutes. At the end of the test, the subject was allowed to reunite with the herd at pasture. Each animal was tested only once.

2.3. Behavioural recording and scoring

To avoid influencing the behavioural responses of the lambs, their behaviour was recorded by an experimenter hidden in a tent placed in front of the metal pen at a distance of 2.5 m. Behavioural observations were made indirectly by video recording, using a GoPro Hero9 Black (GoPro Inc, CA, USA) camera installed outside the tent and that was turned on before each isolation test was performed. Videos were watched continuously during the 8min test duration, and the behavioural patterns of the isolated lambs were catalogued in an ethogram created using Boris software (Friard and Gamba, 2016), including both state and point events (table 1). The videos were scored by a single trained experimenter.

Table 1. Ethogram of the behaviours, postures and social interactions assessed during the isolation tests and their description.

Scored behaviour	Behaviour type	Description
Bodily activation		**Static (standing + lying down) and dynamic (walking + running) behaviours**
Standing	State	The lamb stands on its four legs and remains still for at least two seconds
Walking	State	The lamb slowly moves forward or backward
Running	State	The lamb moves forward with a fast gait, lifting one limb before the other is supported
Head movements	Point	All changes (i.e., fast and slow) in the position of the lamb's head in any direction
Bar head touching	Point	The lamb sticks its head between the bars of the pen or hits them
Jump/rearing on the hind legs	Point	The lamb is off the ground with all legs raised/the lamb is standing on its hind legs
Lying down	State	The lamb is in sternal or lateral decubitus (abdomen in contact with straw bedding)
Ears position		**The position of the lamb's ears in relation to the orientation of the auricles**
Open	State	The inner and outer sides are not visible, the inner side is parallel to the floor, the observer can see the full ears
Flat	State	The inner sides are visible by an observer placed in front of the animal
Hidden	State	The inner sides are not visible, the outer sides are visible at the root of the ear, the ears cannot be fully seen by the observer
Asymmetric	State	One inner side visible, one not
Unidentifiable	State	The position of the lamb’s ears cannot be classified because one of them at least is not visible
Tail position	State	**The position of the lamb's tail**
Upward	State	The lamb raises its tail up above a 45-degree angle
Down	State	The lamb has its tail down, below a 45-degree angle

Arched upward	State	The lamb keeps its tail arched over its rump
Shaking	State	The lamb moves its tail rapidly in any direction
At 45 degrees	State	The lamb has its tail raised at a 45-degree angle
Unidentifiable	State	The position of the lambs’ tail is not visible
Vocalisation	Point	**The lamb is bleating**
Open mouth	Point	The lamb bleats with its mouth open or begins to bleat with its mouth closed and ends with its mouth open
Closed mouth	Point	The lamb bleats with its mouth closed
Social interactions	Point	**Interaction with one or more members of the group through the grid during partial isolation**
Social licking	Point	The lamb is licking one member of the group
Social sniffing	Point	The lamb is sniffing one member of the group
Proximity to the audience	State	Proximity to the closest group members. A lamb was considered in proximity if the distance between it and at least one member of the audience was less than one ewe's length
Sheep to sheep contact	State	The isolated lamb is in body contact with another/others sheep
Excreting behaviours	Point	**The lamb is urinating or defecating**
Urinate	Point	The lamb is urinating
Defecate	Point	The lamb is defecating

Note: behaviour scoring was normalised by the time the sheep was visible

The position of the ears and the tail were recorded as state events, in order to calculate not only the occurrence of the postures, but also the time that the lamb spent with its ears and tail in a particular position. Two additional behaviours were calculated from the catalogued behaviours, namely the number of body position changes (defined as the sum of standing, walking, running and lying down behaviours) and a 'Social score' expressed during the partial isolation phase, calculated as the sum of the number of social sniffing and licking, the number of body contacts with at least one audience member (sheep to sheep contacts) and the number of subjects in contact. Behaviour scoring was normalised by the time the sheep was visible as follows: both state and point behaviours were calculated as frequency of occurrences, by dividing their number of occurrences by the total time the animal was visible; state events were also expressed as percentage of the total time when the lamb was visible by dividing their duration by the total time the animal was visible.) The ear and tail positions were excluded from further behavioural analysis due to the low percentage of time they were visible during both phases of the isolation tests (15.1 ±19.0% and 33.1±21.9% for ears and tail, respectively). The biometric characteristics of the animals were assessed by visual inspection of the videos, as it was not possible to weigh the animals. Lambs were considered "small" if their height was less than 52 cm and "big" if their height was greater than 52 cm

2.4 Acoustic recordings and analyses

Vocalizations produced by the isolated lambs were recorded with an omnidirectional microphone (Sennheiser MKH20) placed at approximately 3.4 m in front of and 2.4 m above from the isolated lambs. The microphone was connected to a recorder (Marantz PMD661, sampling frequency: 44100 Hz and 16-bit WAV file format). Calls were then visualised and automatically detected in the audio files and automatically pre-labelled as high bleats, emitted by the isolated lamb and characterized by a good quality by using the R-package *SoundChunk* (Villain and Renaud-Goud, 2023). The sounds associated to each label were then manually inspected in Audacity to verify their type (bleating or other sounds, open mouth bleat (high bleat=HB) or closed mouth bleat (low bleat=LB)), quality (good if no overlapping sounds were present)). Finally, the inspection of the Signal to Noise Ratio (SNR) revealed a 10.1 ± 0.56 dB difference between a sample (n = 464) of background noises and the analysed calls. Only HB were included in the statistical analysis, as the incidence of LB was very low (22 calls out of a total of 1280 calls). Of the HB, only the ones with a good quality were considered for further analyses (n = 409), and their acoustic features (table 2) were extracted using the "extract_chunk_features_dir" function (*SoundChunk* package; Villain and Renaud-Goud, 2023). The extraction of the acoustic features was computed as follow (Table

2). The sound was bandpass filtered between 150 and 6000 Hz using “hanning” window type (FFT window length = 1024 pts and overlap = 50%). The choice of this bandpass filtering was based on the characteristics of the mean spectrum. The duration of the calls was determined using an amplitude threshold of 3% on the smoothed envelop (window length = 4000 pts, overlap = 90%). The extracted acoustic features related to the mean spectrum were the Median, Inter Quartile Range (IQR) and the Wiener entropy (sfm). The Dominant Frequency (figure 1) was computed on a sliding temporal window (FFT window length = 1024 pts and overlap = 50%) with a 10% amplitude threshold, by searching the loudest frequencies in each window. The fundamental frequency (f_o) was detected by filtering the calls with a frequency range of 150-350 Hz and by searching the loudest frequencies within this band (figure 1).

Finally, formant analysis was performed by searching for three main formants between 350 and 6000 kHz. Based on the detection of three peaks on a mean spectrum computed with FFT window length of 256 pts, the mean spectrum was split into three frequency intervals. For each frequency interval, the calls were filtered to extract the mean dominant frequency over the duration of the call and the number of predicted formants for each frequency interval.

Table 2. Acoustic features extracted from the high bleats and used for acoustic analysis

Acoustic feature	**Unit**	**Description**
Duration	s	Total duration of the vocalisation
The Median	Hz	Frequency below which 50 % of the energy is contained
Inter Quartile Range (IQR)	Hz	Frequency range between the third and the first quartile (indicates the bandwidth)
Wiener entropy (sfm)	-	Ratio of the geometric mean to the arithmetic mean of the spectrum (0: pure tone; 1: random noise)
Dominant frequency mean	Hz	Mean of the dominant frequency, computed per FFT window over the duration of the vocalization
Dominant frequency sd	Hz	Standard deviation of the dominant frequency, computed per FFT window over the duration of the vocalization
Relative Amp. F2*	dB	Relative amplitude of the second formant
Relative Amp. F3*	dB	Relative amplitude of the third formant
Mean F1	Hz	Mean frequency value of the first formant
Mean F2	Hz	Mean frequency value of the second formant
Mean F3	Hz	Mean frequency value of the third formant
Predicted formants**	N	Number of predicted formants, based on LPC coefficient computation
f_o mean	Hz	Mean of the fundamental frequency of the vocalization over the duration of the vocalisation, computed per FFT window over the duration of the

		vocalization
f_o sd	Hz	Standard deviation of the fundamental frequency of the vocalization, computed per FFT window over the duration of the vocalization

** relative amplitude= normalized amplitude (the amplitude of the bleats has been reduced or scaled so that they fall between 0 and 1; for each sound, the loudest amplitude has been set to 1 and the other sounds have been normalised between 0 and 1);**In sheep,three formants are expected below 6000 Hz*

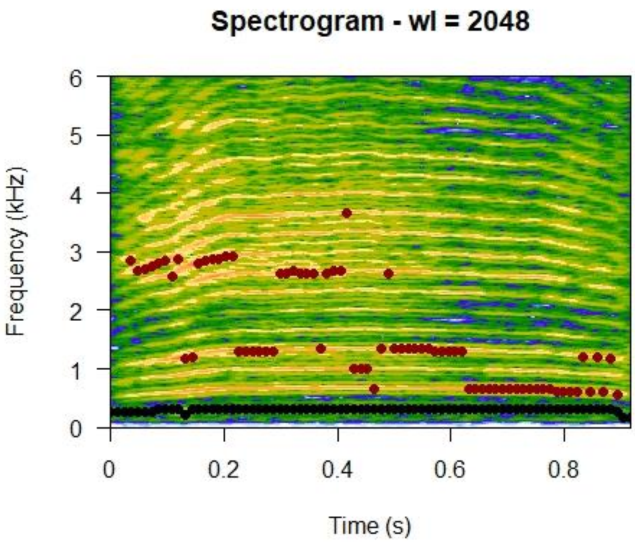

Figure 1. Example of detection of dominant frequencies (red dots) and fundamental frequency (f0; black dots) *in the spectrogram of one sheep bleat using SoundChunk*

2.5 Thermal profiles

Thermal images of the lamb in partial and full isolation were taken with a Hikmicro G60 by using the following parameters: emissivity = 0.98, reflected temperature = 25 °C and distance = 4m. To avoid influencing the subject's emotional response, the experimenter took the pictures while hidden in the tent. The thermal images (n = 3010) were manually inspected to determine their quality (out of focus images were considered of poor quality and then discarded) and the number of areas of the lambs' face (i.e., ears, eyes and muzzle) was also counted. Nine hundred and eighteen good quality images were labelled using Hikmicro Analyser software. The areas of the face where the measurements were taken were the eyes, ears and muzzles, from which the thermal matrices were extracted. The labelling also took into account the posture of the lamb in the image (front, profile and back) and the type of contouring of the facial area (full or partial). The maximum temperature of each areas of the face was used for statistical analysis, to avoid the impact of the size of the area on the computation of the mean temperature. Finally, a delta time value was calculated between the time when the thermal images were taken and the start of each isolation phase, in order to have an information about the temporal distribution of the thermal images during the phases. Measurements collected on the ears and muzzle were not retained for further analysis due to abnormally low values of maximum temperatures of these facial areas, probably due to evapotranspiration phenomena or the presence of wool on the outer side of the lambs' ears.

2.6 Statistical analysis

All statistical analyses were all performed in R Studio (version 4.2.3, released 2023-03-15).

2.6.1 *Behavioural analysis*

A Principal Component Analysis (PCA) was first performed to generate behavioural scores and reduce the number of response variables, by using the 'dudi.pca' function from the 'ade4' R package (Dray and Dufour, 2007). Based on a previous PCA performed on all the behaviours scored, the following behaviours were included in the final PCA to generate behavioural scores: the Jump/rearing on the hind legs rate, the total time of Static behaviours (behavioural category created by aggregating standing and lying down behaviours) in percent, the total time Walking in percent, the total time Running in percent, the rate of Body position changes, the number of Bleats produced, the rate of Bar head touching and the rate of Head movements.. The normality of behavioural parameters was visually inspected using histograms. As a result, the time the lambs spent in static positions and walking was transformed to logarithm (+1), and the jump rate and vocal dynamic were square root transformed before being included in the PCA, in order to approximate a normal distribution. All principal components (PCs) with an eigenvalue greater than one were retained for further analyses.

The effect of the phase of the test, the individuals' sociability and the biometric characteristics on the lambs' behavioural response to isolation was investigated using a Linear Mixed-Effects Model (LMER) ('lmer' function from 'lme4' R package; Bates et al., 2015). Composite behavioural scores were used as the response variable in the model, after checking their normal distribution using histograms. The following fixed factors were included in the model: the phase of the test (partial or full), the social score (low or high) and lamb size (small or big), as well as two-by-two interactions between the phase of the test and the social score, between the phase of the test and the lamb size and between the social score and the lamb size (Model 1). Moreover, the social score was considered in interaction with the size of the lamb. Lastly, the individual identity of the subject was included in the model as a random effect to control for repeated measurements.

*Model 1: Behavioural score ~ phase of the test*social score + phase of the test*lamb size + social score*lamb size +(1|subject)*

2.6.2 Acoustic analysis

Similarly as for the behavioural variables, a composite acoustic scores were computed to describe the general structure of the vocalisation while decreasing the number of variables. A PCA was performed on the set of acoustic features listed in table 2 by using the 'dudi.pca' function. from the 'ade4' R package (Dray and Dufour, 2007). The normality of acoustic features was visually inspected by means of histograms and all principal components (PCs) with an eigenvalue greater than one were retained.

The comparison between the calls produced in partial and in full isolation by the same individuals was investigated with a Linear Mixed-Effects Model (LMER) ('lmer' function from 'lme4' R package; Bates et al., 2015). The following fixed factors were included the model: the phase of the test (partial or full) and the lamb size (small or big) as well as two-by-two interactions between the phase of the test and the lamb size (Model 2). Lastly, the individual identity of the subject was included in the model as a random effect to control for repeated measurements. Since not all lamb produced calls during the partial isolation, this analysis could be performed on a reduced dataset composed on the 6 animals that produced calls during both phases of the tests (N = 27 calls during partial isolation, and N = 128 during the full isolation). Considering the number of observations, the social score could not be implemented in the model.

*Model 2: acoustic score ~ phase of the test*lamb size + (1|Subject), data = data)*

2.6.3 Thermal images analysis

To study the effect of effect of the phase of the test, the temporal distribution of the thermal images during the phases, the biometric characteristics of the individuals and their sociability on the maximum eyes temperature, we used the same model as in the behavioural analysis. The following fixed factors were included in the model: the phase of the test (partial or full), the social score (low or high) and the lamb size (small or big); moreover, the time at which a thermal image was taken during the studied phase (i.e., delta from phase start) was also included as a scaled covariate. The model also considered two-by-two interactions between the phase of the test and the delta from phase start, between the phase of the test and the social score, between the phase of the test and lamb size and between the social score and the lamb size. Lastly, the individual identity of the subject was included in the model as a random effect to control for repeated measurements (Model 3).

*Model 3: maximum eyes temperature ~ phase of the test*delta from phase start + phase of the test*social score + phase of the test*lamb size + social score*lamb size +(1|subject)*

A second model implemented for the thermal image analysis used a Coefficient of Reaction as the response variable, computed with the following formula:

CR=(T - baseline)/baseline

where*:*

T = maximum eyes temperature in partial and full-isolation

baseline = average of maximum eyes temperature in partial isolation

Model 4 was built with the aim to test the effect of the behavioural scores on the Coefficient of Reaction. The following fixed factors were included in the model: the social score (low or high) and the lamb size (small or big) as well as two-by-two interactions between the behavioural score and the social score, between the behavioural score and the lamb size and between the social score and the lamb size. Lastly, the individual identity of the subject was included in the model as a random effect to control for repeated measurements.

*Model 4: CR ~ behavioural score*social score + behavioural score* lamb size + social score* lamb size +(1|Subject)*

2.6.4 Vocal, behavioural and physiological analysis

To evaluate the relationship between the acoustic structure and behavioural and physiological response to isolation, we built a model using the composite acoustic scores as response variables (Model 5). In this model a different Coefficient of Reaction was considered from that computed in model 3, in order to have a delta of ocular temperature passing from the partial to full isolation for each lamb. In this case, a single CR was calculated per lamb and per phase, by considering CR = 0 if the call was emitted in partial isolation and by calculating CR in full isolation with the formula:

CR = (T°-baseline)/baseline

where*:*

T = mean of maximum eyes temperature in full isolation

baseline = mean of the maximum eyes temperature in partial isolation

The following fixed factor was included in the model: the lamb size (small or big), as well as two-by-two interactions between the behavioural score and the lamb size, between the behavioural score and the Coefficient of Reaction and between the Coefficient of Reaction and the lamb size. Lastly, the individual identity of the subject was included in the model as a random effect to control for repeated measurements.

*Model 5: acoustic score~ behavioural score*lamb size + behavioural score*CR + CR* lamb size + (1|Subject)*

2.6.5 Model fit and extraction of p - values (for all models)

The distribution of the residuals for each model was assessed by a visual inspection of the plot and the histogram of their residuals. In order to assess the effect of the fixed factors, a type-II analysis-of-variance was performed using the 'Anova' function from the 'car' R package (Fox and Weisberg, 2018). When significant interactions were found, pairwise comparisons were computed using 'emmeans' and 'emtrends' function from 'emmeans' R package (Lenth, 2016), with Tukey correction for factors and 'emtrends' function for interacting covariates. When significant interactions were found in model 5, comparisons were carried out by fixing some values of the covariate CR (mean = 0.01; mean of CR – sd of CR = -0.05; mean of CR + sd of CR = 0.07). Positive values (i.e., 0.01 and 0.07) indicated an increase in the maximum eyes' temperature from partial to full isolation and the negative value (i.e., -0.05) indicated a decrease.

3. Ethical statement

This study was approved by the Animal Ethics Institutional Review Board at the Faculty of Health and Medical Sciences of the University of Copenhagen (official number: 023-03-BehaVEco-007A). As social isolation induces a negative affective state in the lambs, both isolation phases lasted only 4 minutes each. The isolation took place in a familiar environment, and any negative effect of this procedure on the sheep was not expected to last beyond the end of the test. Five isolation trials were interrupted because the lamb being tested escaped.

4. Results

4.1 Behavioural response of lambs to partial vs full isolation

The Principal Component Analysis (PCA) performed on the behaviours resulted in two principal components with an eigenvalue greater than 1. The first principal component (PC1) explains 58.4% of the variance in the data, and a second component explains 14.9% of the variance. On the basis of the loadings of the behaviours on the PCs (table 3), more positive scores of PC1 indicated more calls produced, more time spent running, changing body positions, jumping and performing bar head touching, and less time spent in static behaviours (standing + lying). More positive scores of PC2 on the other hand, indicated less time spent walking. Overall, therefore, increases in both PC1 and PC2 indicated an increased level of arousal, and are thus used as ‘Activation Scores’.

Table 3. Results of the Principal Component Analysis performed on the behavioural dataset, showing the parameters loadings on PC1 and PC2 (relative contribution for each behaviour to the PCs). The high parameters loading (i.e., > 50 or < -50) are in bold

Behavioural parameters	**PC1**	**PC2**
Jump rate	**70.7**	0.01
Percent of total time in in static behaviours (log1)	**-65.5**	-0.01
Percent of total time walking (log1)	5.08	**-88.3**
Percent of total time running	**76.7**	11.5
Rate of body position changes	**74.3**	-2.17
Vocal dynamic (sqrt)	**80.1**	4.91
Rate of bar head touching	**52.0**	0.58
Rate of head movements	43.2	-12.0

Effect of the phase of the test and biometric characteristics on behavioural response to isolation

Behavioural PC1 was significantly affected by the interaction between the phase of the test and the biometric characteristic of the lamb (Model 1: Chisq = 3.95; df = 1, p = 0.047; figure 2). Further two-by-two comparisons showed that the behavioural response PC1 (Activation Score 1) of both big and small lambs was higher in full isolation than in partial isolation and that PC1 increased to a greater extend in small lambs (t.ratio = 5.35, p = 0.0003; t.ratio = 7.84; p = < 0.0001 for big and small lambs, respectively). This suggests that, in particular small but also big lambs, were more active in full than partial isolation, No significant differences in the behavioural response were found between small and big lambs in partial isolation (t.ratio = 1.36, p = 0.53) and during the full-isolation (t.ratio = -0.85; p =0.83). No evidence of any significant effect of the social score was found for behavioural PC1 (Chisq = 0.19; df = 1, p = 0.66 for the single effect of the social score;

Chisq = 0.94; df =1, p = 0.333 for the interaction between the phase of the test and the social score).

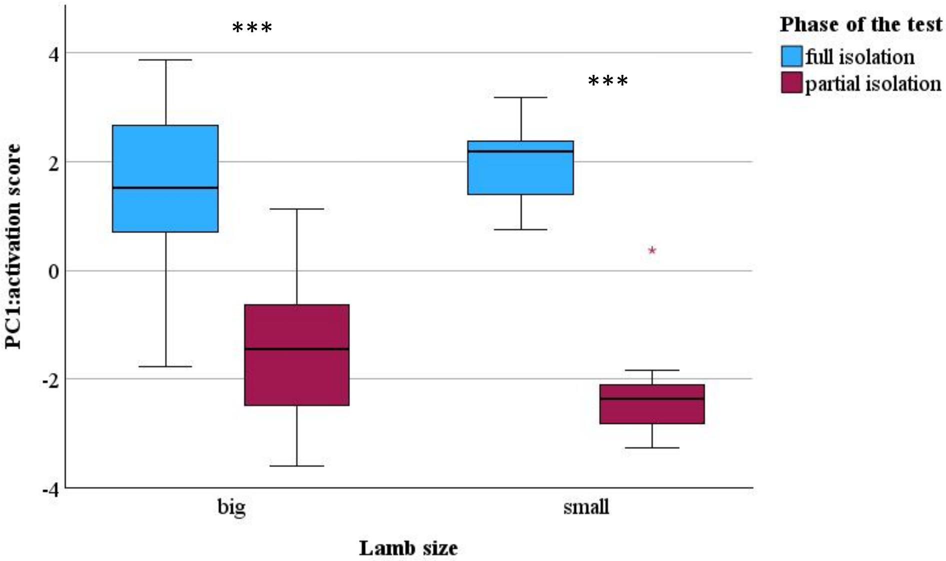

Figure 2. Lambs' behavioural response (Activation Score) as a function of their biometric characteristics and the phases of the test. Box plots show the median (centre line), 25th and 75th percentiles (the bottom and top of the boxes). The length of the boxes is the interquartile range (IQR). Lines extending from the boxes (i.e., whiskers) extend 1.5 times the IQR. Coloured asterisks indicate outliers. ***Statistical table (Anova table, posthoc tests and model estimates are available in supplementary tables S1, S2, S3 respectively, model 1)***

No evidence of any significant effect of the phase of the test, the biometric characteristics of the lamb, nor the social score was found for behavioural PC2 (Chisq = 2.79; p = 0.095 for lamb size supplementary materials, table S4). Only behavioural PC1 was therefore used as Activation Score for further analysis.

4.2 Acoustic structure of vocalizations in partial and full isolation: relationship with bodily activation

Acoustic scores:

The acoustic analysis was performed on a dataset of 409 high bleats, of which 382 were emitted during the full isolation and only 27 during the partial isolation. The Principal Component Analysis performed on the acoustic data showed four components that had an eigenvalue higher than 1 (PC1-PC4). PC1 explained the 42.7% of the total variance and PC2 13.0%. According to the loadings of the acoustic features on the PCs (Table 4), more positive PC1 scores indicated calls with a higher median of the mean spectrum (Hz), a higher Wiener entropy (sfm), a higher mean dominant frequency (Hz) and dominant frequency sd and a higher mean of the second and third formants (Hz). Therefore, as PC1 scores increased, calls become noisier (> Wiener entropy) and were characterised by higher frequencies. No feature loaded highly on PC2, which this PC was thus not retained for further analysis. More positive scores of PC3 indicated calls that were characterized by a lower value of Inter Quartile Range, indicating that Q25 and Q75 are closer together, so that the energy is concentrated in a small part of the call (i.e., smaller bandwidth). Finally, high values of PC4 indicated calls that were longer in duration.

Table 4. Results of the Principal Component Analysis performed on the acoustic dataset giving the features loadings on PC1, PC2, PC3 and PC4 (relative contribution for each behaviour to the PCs). The high features loading (i.e., > 50 or < -50) are in bold.

Acoustic features	**PC1**	**PC2**	**PC3**	**PC4**
Duration	6.21	7.93	-7.78	**59.3**
Median	**84.3**	-5.12	1.90	-0.03
Inter Quartile Range (IQR)	22.6	2.86	**-60.5**	-2.63
Wiener entropy (sfm)	**64.5**	-1.00	-9.57	-4.70
Dominant frequency mean	**64.0**	-9.60	16.5	0.27
Dominant frequency sd	**55.9**	-10.1	-1.26	-0.26
Relative Amp. F2	27.4	-3.34	20.6	-0.15
Relative Amp. F3	36.8	-41.8	-0.06	0.28
Mean F1	47.6	21.3	8.55	2.92
Mean F2	**51.3**	23.5	7.65	0.35
Mean F3	**65.3**	16.1	0.84	-0.41
Predicted formants	11.4	-14.4	-12.8	0.75
f_o mean	48.9	1.69	-11.2	2.03
f_o sd	11.1	22.8	-0.41	-28.6

The following analyses were performed on a subset of the dataset by considering only those lambs that produced bleats in both partial and full isolation (n = 155 calls produced by 6 lambs, 27 calls produced in partial isolation and 128 calls produced in full isolation). Acoustic PC1 was significantly affected by the phase of the test (model 2: Chisq = 4.47; df = 1; p = 0.035; figure 3); its scores were higher, indicating noisier and higher frequency calls, in full isolation compared to partial isolation (estimated mean 95% [CI] = 0.01 [-1.84 - 1.86] and -0.393 [-2.21 – 1.42] for partial and full isolation, respectively).

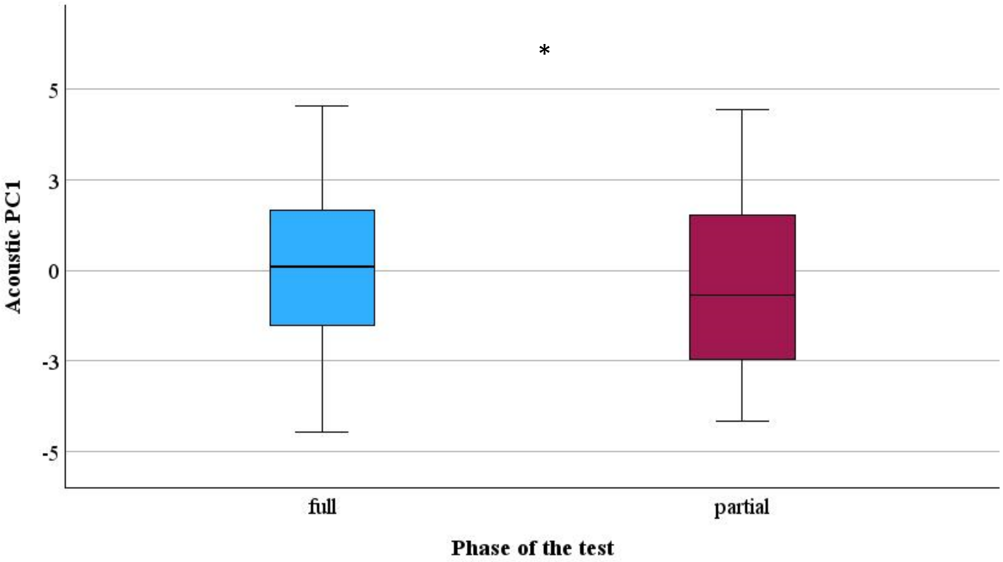

Figure 3. Acoustic PC1 as a function of the phase of the test. Box plots show the median (centre line), 25th and 75th percentiles (the bottom and top of the boxes). The length of the boxes is the interquartile range (IQR). Lines extending from the boxes (i.e., whiskers) extend 1.5 times the IQR. Coloured asterisks indicate outliers. ***Statistical table (Anova table and model estimates are available in supplementary tables S5 and S6 respectively, model 2)***

Acoustic PC3 was significantly affected by the interaction between the phase of the test and the size of the lamb (model 2: Chisq = 4.20; df =1; p = 0.040; figure 4). Further two-by-two comparisons revealed more positive Acoustic PC3 scores in small lambs in full isolation, suggesting calls with smaller bandwidth (IQR), than in partial isolation, (t.ratio = 3.69; p = 0.002). There was no evidence of an effect of the interaction between the phase of the test and the size of the lambs on Acoustic PC3 for big lambs (t.ratio = 0.96; p = 0.77).

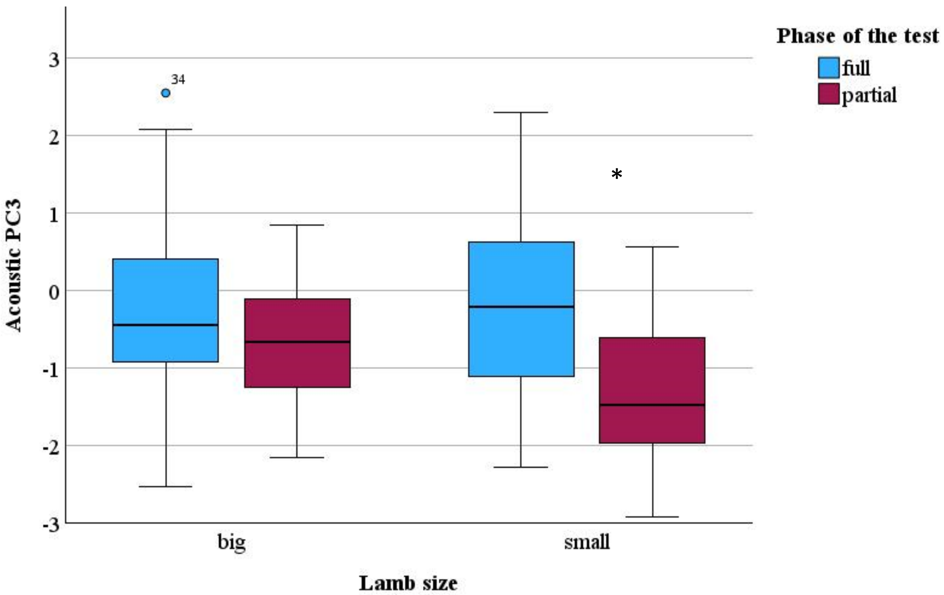

Figure 4. Acoustic PC3 in function of biometric characteristics and the phases of the test. Box plots show the median (centre line), 25th and 75th percentiles (the bottom and top of the boxes). The length of the boxes is the interquartile range (IQR). Lines extending from the boxes (i.e., whiskers) extend 1.5 times the IQR. Coloured asterisks indicate outliers. ***Statistical table (Anova table, posthoc tests and model estimates are available in supplementary tables S7, S8, S9 respectively, model 2)***

Acoustic PC4 was significantly affected by the phase of the test (model 2: Chisq = 16.70; df =1; p < 0.0001; figure 5). Its scores were lower, suggesting shorter calls, in full than partial isolation (estimated mean 95% [CI] = - 0.55 [-1.38 – 0.29] and - 0.08 [-0.73 – 0.90] for partial and full isolation, respectively).

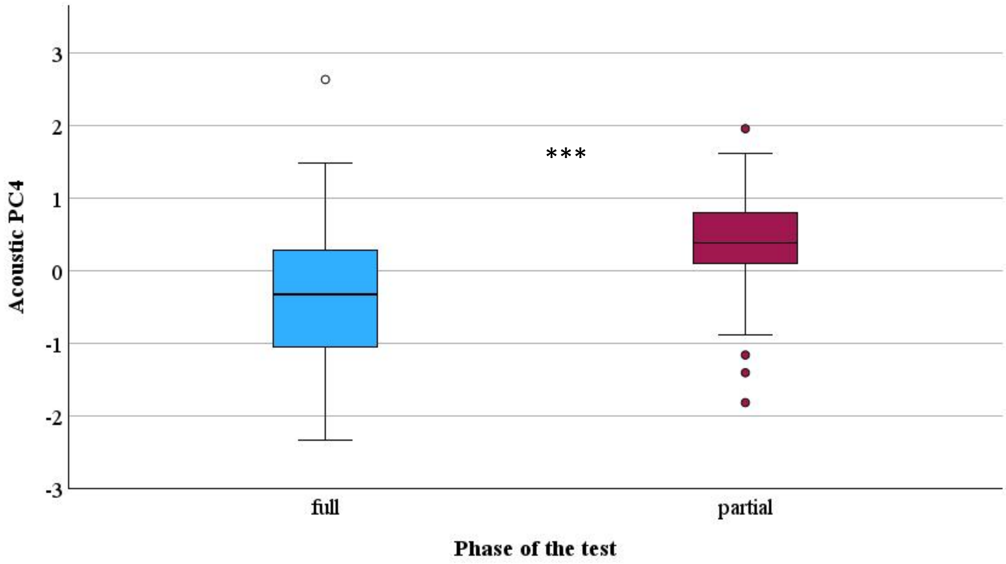

Figure 5. Acoustic PC4 in function of the phase of the test. Box plots show the median (centre line), 25th and 75th percentiles (the bottom and top of the boxes). The length of the boxes is the interquartile range (IQR). Lines extending from the boxes (i.e., whiskers) extend 1.5 times the IQR. Coloured asterisks indicate outliers. ***Statistical table (Anova table and model estimates are available in supplementary tables S10, S11 respectively, model 2)***

4.3 Thermal profile and reaction to full isolation

Labelling of thermal images taken during partial and full isolation allowed the creation of a dataset consisting of 770 eyes. Of these, 481 were acquired during partial isolation and 289 during full isolation.

4.3.1 Effect of the phase of the test and biometric characteristics on physiological response to isolation

Maximum eye temperature was significantly affected by the interaction between the phase of the test and lamb size (model 3: Chisq = 26.36; p < 0.0001; figure 6). Further two-by-two comparisons revealed that the maximum eye temperature of the small lambs was higher in full isolation than in partial isolation (t.ratio = 6.70; p < 0.0001). On the other hand, the maximum eye temperature of big lambs in full isolation was not significantly different from that in partial isolation (t.ratio = -0.25; p = 0.995). Moreover, the maximum eye temperature was higher for big compared to small lambs both in partial and full isolation (t.ratio = 5.20; p = 0.0004 and t.ratio = 3.11; p = 0.029, respectively).

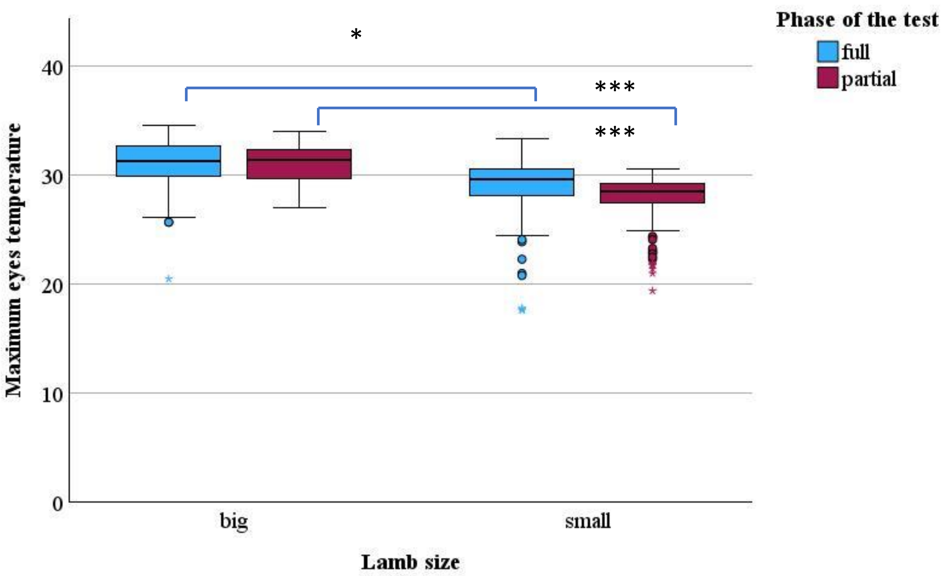

Figure 6. Maximum eyes temperature as a function of the phase of the test and the biometric characteristics of the lambs. Box plots show the median (centre line), 25th and 75th percentiles (the bottom and top of the boxes). The length of the boxes is the interquartile range (IQR). Lines extending from the boxes (i.e., whiskers) extend 1.5 times the IQR. Coloured asterisks indicate outliers. ***Statistical table (Anova table, posthoc tests and model estimates are available in supplementary tables S12, S13, S14 respectively, model 3)***

The maximum eye temperature was marginally affected by the delta from the start of the phase (Chisq = 4.40, p = 0.04, mean estimate = 29.4; CI = 28.7 - 30.2), showing a slight increase between time from 0 min to 4 min. No evidence for an effect of the social score was found on maximum eyes temperature (Chisq = 2.66; df = 1, p = 0.103 for the single effect of the social score, Chiq = 0.005; df = 1, p = 0.94 for the interaction between the phase of the test and the social score and Chisq = 0.69; df = 1, p = 0.40 for the interaction between the social score and the lamb size).

4.3.2 Effect of the Activation score and the biometric characteristic of individuals on the Coefficient of Reaction

The Coefficient of Reaction (CR) was significantly affected by the interaction between Activation Score and lamb size (model 4; Chisq = 15.17; p < 0.0001; figure 7). The Coefficient of Reaction showed a small but significant increase with behavioural response (i.e., Action Score or behavioural PC1) for small lambs (trend of the linear slope = 0.01; CI = 0.01 – 0.01), and a non-significant (based on the values of the confidence interval) change for big lambs (trend of the linear slope = -0.000344: CI = -0.005 – 0.004; figure 4).

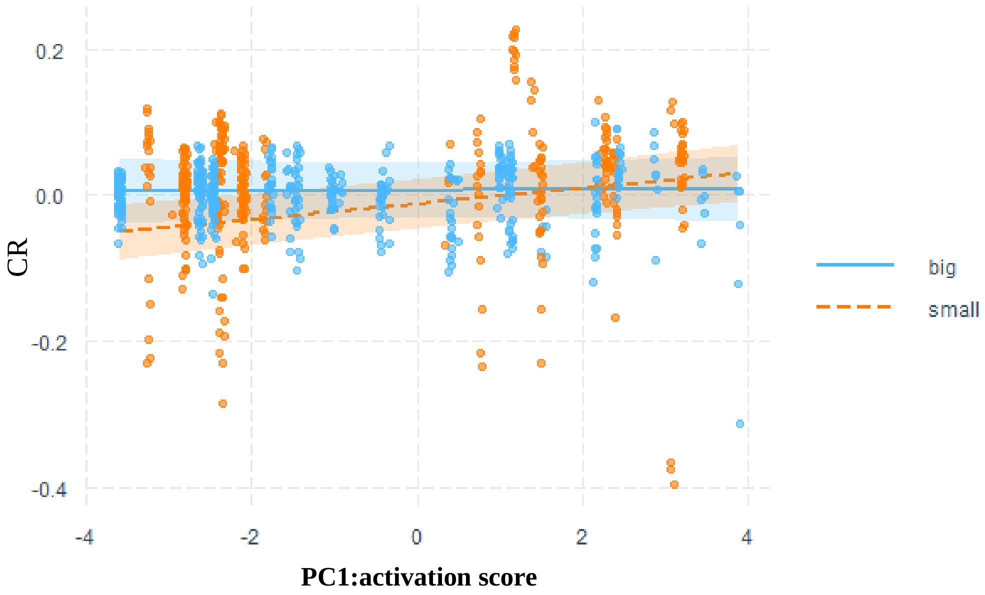

Figure 7. Coefficient of reaction as a function of PC1:activation score and the biometric characteristics of the lambs. ***Statistical table (Anova table, posthoc tests and model estimates are available in supplementary tables S15, S16, S17 respectively, model 4)***

4.4 Relationships between bleats acoustic structure and behavioural and physiological response to isolation

Acoustic PC1 was significantly affected by the interaction between the behavioural score and CR (model 5: Chisq = 4.20; df = 1; p = 0.04; figure 8). The acoustic PC1 increased with the behavioural score (PC1:Activation score), but only when the Coefficient of Reaction was positive (trend of the linear slope = 0.28; CI = 0.10 - 0.46 with 0.01; trend of the linear slope = 0.87; CI = 0.15 - 1.59 with 0.07), suggesting and increase in the frequencies and noisiness of the calls with the bodily activation and with the ocular temperature. The trend of the slope of the Acoustic PC1 as a function of the Activation score when CR was low (negative) was not significant, based on the values of the confidence interval (mean trend = -0.31: -0.85 - 0.22; figure 8).

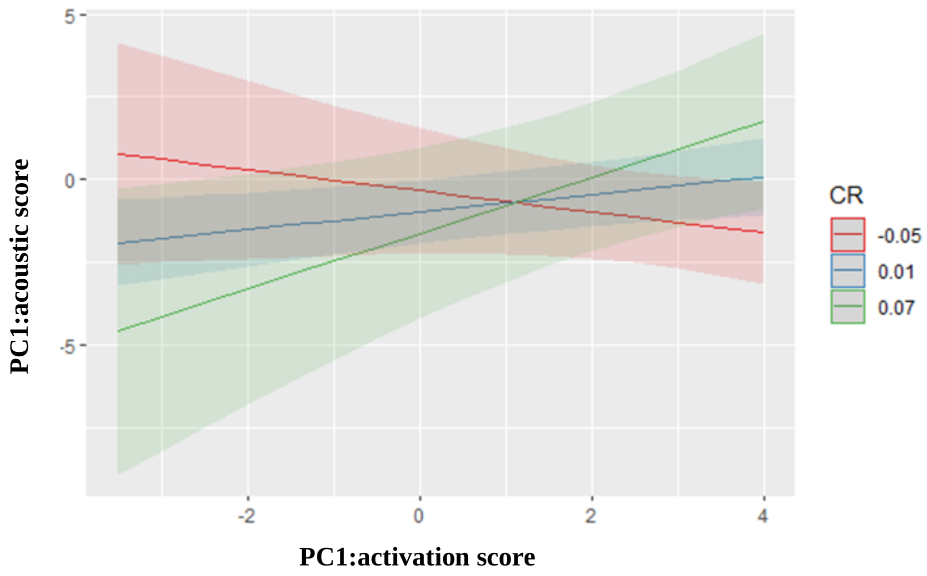

Figure 8. Acoustic PC1 in function of the lambs' bodily activation (PC1:Activation score) and the Coefficient of Reaction at fixed point (mean – SD, mean, meand +SD). ***Statistical table (Anova table and model estimates are available in supplementary tables S18, S19 respectively, model 5)***

Acoustic PC1 was significantly higher in small lambs compared to big lambs (single effect of lamb size, Chisq = 7.63; p = 0.01), regardless of Activation score or thermal CR, meaning that these individuals produced more noisy bleats with higher frequencies (statistical table: Anova table and model estimates are available in supplementary tables S18, S19 respectively, model 5).

Acoustic PC3 was significantly affected by the interaction between the behavioural score and the Coefficient of Reaction (model 5: Chisq = 5.55; df = 1; p = 0.02; figure 9). Further post-hoc comparisons revealed a significant increase of acoustic PC3 with the behavioural score, but only for high values of CR (CR fixed at mean + SD, trend of the linear slope = 0.72; CI = 0.11 - 1.33; CR fixed at mean, trend of the linear slope = 0.01; CI = -0.02 – 0.35; CR fixed at mean – SD, trend of the linear slope = -0.39; CI = -0.83 – 0.05; figure 9).

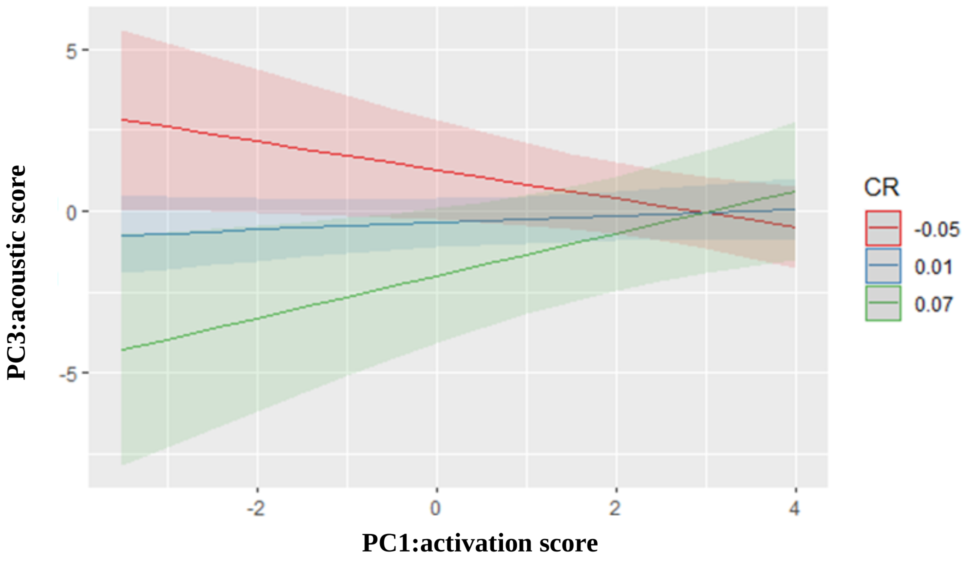

Figure 9. Acoustic PC3 in function of the lambs' bodily activation (PC1:Activation score) and the Coefficient of Reaction at fixed point (mean – SD, mean, meand +SD). ***Statistical table (Anova table and model estimates are available in supplementary tables S20, S21 respectively, model 5)***

Acoustic PC4 was significantly affected by the interaction between the behavioural score and the biometric characteristics of the lamb (Chisq = 4.12; df = 1; p = 0.04; figure 10), regardless of CR. Further post-hoc comparisons revealed that PC4 decreased as the activation score increased, and this effect was larger for small lambs (trend of the linear slope = -0.47; CI = -0.71 – (- 0.23) than big lambs (trend of the linear slope = -0.19; CI = -0.36 – (0.01); figure 10).

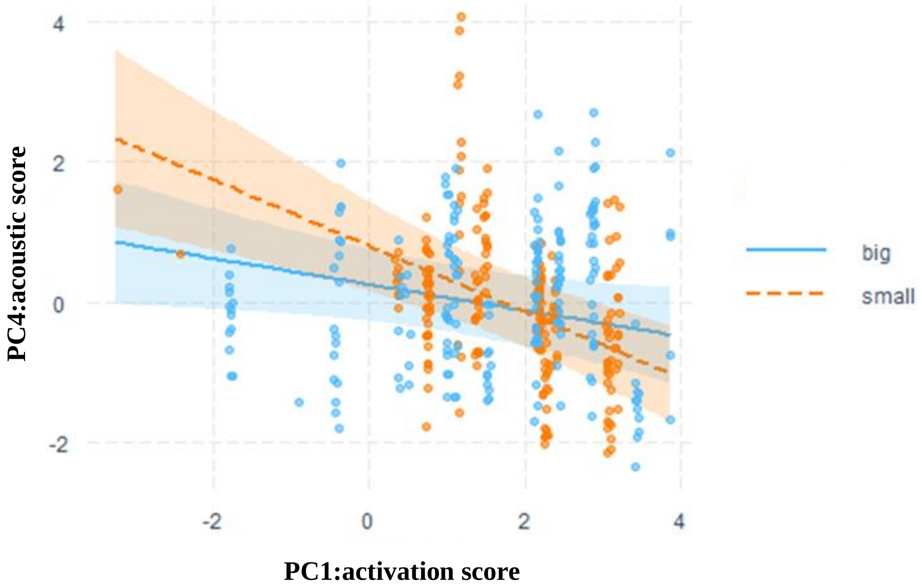

Figure 10. Acoustic PC4 in function of the behavioural score and the biometric characteristics of the lambs. ***Statistical table (Anova table and model estimates are available in supplementary tables S22, S23 respectively, model 5)***

5. Discussion

We investigated the behavioural, vocal and physiological responses of lambs to experimental situations inducing different levels of arousal in order to identify non-invasive indicators of this emotional dimension in the sheep species. We found behavioural and vocal indicators of negative arousal in lambs that changed consistently with increasing arousal. The physiological parameters investigated in the present study did not change consistently with arousal, but showed differences related to the size of the lambs. If a relationship is found between physiological parameters with some acoustic features and the behavioural response, further research is needed to investigate the possibility of using thermography as a technique to detect any change in the intensity of the response to a negative stimulus. The identification of arousal indicators is important as it allows the stress experienced by animals in negative situations to be reduced (Boissy et al., 2007). However, more tests should be conducted in positive states to identify indicators of positive arousal.

5.1 Behavioural response to negative arousal

In this study, lambs spent more time running and less time in static behaviours (i.e., standing and lying) performed more jumps, changes in body position and bar head touching and produced more calls, suggesting a higher arousal level, during full isolation than during partial isolation. Moreover, a greater behavioural response to isolation was observed for small lambs than big lambs. An increase in locomotor activity can be seen as a behavioural response to fear and anxiety induced by social isolation in sheep (Vandenheede et al., 1998), and resulting in escape attempts (Siebert et al., 2011). The fight-or-flight response is enacted by individuals in negative situations characterised by high levels of arousal, because it allows them to respond quickly to threats to survival and fitness (Dawkins, 1998). Therefore, an increase in the individual's bodily activation would be an indication that they are in a state of high emotional arousal. Increases in locomotor activity (head movements, moving; Briefer et al., 2015a) and escape attempts (jumping and rearing (i.e., standing on hind legs); Siebert et al., 2011) are also observed in goats as levels of emotional arousal increase. The predominant production of high (open-mouth) vocalisations has been observed in sheep in response to stress due to separation from offspring and social isolation (Dwyer, 2008; Papadaki et al., 2021), similarly to our findings. We observed an increased production of calls per time unit (faster rate), which can be attributed to increased respiratory rate with increasing levels of emotional arousal (Briefer, 2020). The emission of more calls per time unit with increasing arousal levels occurs consistently in several species, including other livestock (e.g., cattle, Thomas et al., 2001; pigs; Marchant et al., 2001), and is therefore considered a valid indicator of this emotional dimension (Briefer, 2020). In the present study, it was not possible to investigate ear and tail postures as behavioural indicators of emotional arousal, as they were not visible most of the time. Although there is some evidence in the literature that ear and tail postures reflect the emotional state of sheep (ears Boissy et al., 2011; Reefman et al., 2009a and Vögeli et al., 2015; tail Reefman et al., 2009a), to our knowledge, no postures of these body areas have yet been identified as indicative of any emotional dimensions in these animals, and therefore further research is needed on this topic.

5.2 Physiological response to negative arousal

We used the maximum eyes temperature and the Coefficient of reaction to determine the level of arousal triggered by partial and full isolation. We found an increase in the maximum eye temperature of small lambs from partial to full isolation, but not for big lambs. Several studies that have used infrared thermography to assess negative emotions in sheep, including stress, have measured the temperature of the eye (Stubsjøen et al., 2009; Arfuso et al., 2022) or part of it, such as the lacrimal caruncle (Cannas et al., 2018; Comin et al., 2023), lateral canthus, central cornea and medial canthus (Arfuso et al., 2022). Eye temperature and the aforementioned eye regions increased in response to fear (Comin et al., 2023) and stress (Cannas et al., 2018; Arfuso et al., 2024), similar to what was observed in the present study for small lambs. Here, we extracted the thermal matrices that covered the entire ocular area. Arfuso et al (2022) found that the medial canthus ocular region of sheep was the most reliable for monitoring stress, as it is affected by vasodilation and heat dissipation due to exposure of an animal to a stressor. Medial canthus temperature was also found to be positively correlated with serum cortisol concentration in ewes after the exposure to the stressor and with the rectal temperature in ewes before and after stressor exposure. The absence of an increase in the eyes maximum temperature of the big lambs in full isolation can be explained by the relationship we found between the behavioural and physiological parameters. In fact, we observed a slight but significant increase in the coefficient of reaction with bodily activation in the small lambs and no change in the big lambs. The greater variation in bodily activation observed in small lambs from partial to full isolation could therefore have been detected by thermography, in contrast to the absence of change in big lambs. In addition, we observed a higher maximum eye temperature in big lambs than in young lambs in both partial (i.e., basal temperature) and full isolation. In mammals, body size can influence the metabolic rate and thus the thermoregulatory process (Kleiber, 1947). For instance, in Standardbred trotting horses, a higher basal temperature was observed in young animals (2-3 years) than in adult horses (> 3 years;

Negro et al., 2018). This result is in contrast to the findings of the present study, but it must be emphasised that, to the best of our knowledge, there are no studies explicitly relating eye temperature to animal size within the same species and thus more research in this field is needed.

5.3 Acoustic marker of negative arousal

We found that sheep produced bleats that were characterised by higher frequencies (energy quartiles, formants, fundamental frequency), Wiener entropy, and shorter duration as the level of arousal increased, i.e., from the partial to the full isolation. Furthermore, the relationship between the acoustic, behavioural and physiological parameters showed an increase in the frequency and noisiness of the calls with bodily activation and with a positive Coefficient of Reaction (i.e., an increase in maximum eye temperature from partial to full isolation).

An increase in frequencies has been observed with increases in emotional arousal in many species (goats; Briefer et al., 2015a; piglets, Linhart et al., 2015; horses, Briefer et al., 2015b). Briefer et al., 2015a observed an increase of the f_o mean, but also of other f_o contour features (i.e., f_o end) in goats experiencing high arousal situations (i.e., anticipation of a food reward and food-related frustration vs. control and isolation from conspecifics). The increase of the fundamental frequency with arousal was also observed by Sèbe et al. (2012) in sheep exposed to separation from their offspring. The same finding was obtained by Papadaki et al. (2021) in isolation tests in ewes when they had no lambs under their care (dry period) compared to when they had lamb under their care (suckling period), although the increase in arousal underlying the change in the acoustic structure of bleats was only hypothesized by the authors. The increase in f_o with the intensity of an emotional state may be due to increased subglottal pressure as a result of changes in respiration or laryngeal muscle action/tension or to an increased vocal fold tension (Titze, 1994; Fant, 1960). In this study we also observed a change of bleats energy distribution towards higher frequencies with increasing arousal, as observed in several species (Briefer, 2012; Briefer, 2020). In the goat species, the aforementioned changes were associated to a reduced laryngeal retraction and/or increased pharyngeal constriction with increased arousal (Briefer et al., 2015a). The only available study on the variation of these acoustic features of sheep bleats with arousal level showed opposite results to the present study, i.e., a reduction of energy quartiles (i.e. Q25%, Q50% and Q70%; Sèbe et al., 2012). Engeldal et al. (2013) also found a reduction in energy distribution in vocalizations of sheep subjected to partial than in full isolation (in this study, partial isolation was assumed to induce higher arousal due to proximity to conspecifics and high motivation to restore social contact, contrary to the findings of this study, where full isolation induced greater bodily activation and therefore higher arousal). Although the frequency of lower and higher formants is determined by anatomical features of the vocal tract, i.e., shape and length, respectively (Fant, 1960), high emotional arousal would result in reduced salivation and higher formant frequencies (Scherer, 1986), that could explain our findings. The variation of dominant frequencies in relation to the emotional state of animals has been little studied. An increase of the dominant frequency band with increasing arousal has been observed in monkeys (i.e., in squirrel monkeys with increasing arousal; Fichtel et al., 2001; in Chacma baboon calls to signal the presence of predators) and birds (i.e., in zebra finches under conditions of isolation or after oral administration of corticosterone; Perez et al., 2012).

In this study, lamb calls were found to be noisier as the level of arousal increased, as indicated by an increase in Wiener entropy (sfm). This finding is in line with what was observed in goats by Siebert et al. (2011), who recorded lower entropy in goat calls in complete isolation compared to partial isolation (in this study, complete isolation resulted in lower emotional arousal due to the lack of olfactory and acoustic communication with conspecifics, and therefore to the lack of motivation for social reunion).

The increase in frequencies, fundamental frequency, formants and noisiness of calls observed in small lambs compared to big lambs in the present study was due to anatomical differences and not to a different level of arousal experienced by the lambs.

The interquartile range (IQR) did not show a consistent variation with the level of arousal, only decreasing with emotional intensity in small lambs. Despite this, IQR was observed to decrease with the level of bodily activation when considering the highest Coefficient of Reaction (i.e., 0.07), suggesting a possible effect of arousal on this acoustic feature. Although an increase in IQR with increasing arousal was recorded in some animal species such as piglets, Linhart et al., 2015; Illman et al., 2013)), an opposite trend was observed for this feature in ewes (Sèbe et al., 2012). The inconsistency of the results obtained in this study suggests the need for further research into the variation of this feature with the emotional arousal in sheep.

We observed the production of shorter calls passing from the partial to the full isolation. Although there was a negative relationship between the duration of vocalisations and the level of bodily activation, with a greater decrease for small lambs, the duration of vocalisations did not show a relationship with the Coefficient of reaction. The duration of vocalisations is the only acoustic feature that changes consistently as emotional valence varies across species, and has therefore been suggested as an indicator of this emotional dimension more than of arousal (Briefer, 2020). Nevertheless, a review by Zimmermann et al. (2013) found some evidence for an increase in the duration of vocalisations as the level of arousal increased. In our study, there was insufficient evidence that call duration decreased with arousal. However, the emotional intensity elicited by full isolation may have led to the production of calls of shorter duration because they are quicker to produce and can be repeated rapidly, as demonstrated by the increase in the vocal rate.

No effect of the individual sociability (i.e., Social score) was observed in response to isolation. This contradicts our *a priori* hypothesis that more sociable individuals would experience higher emotional arousal in full isolation than less sociable animals. However, social isolation may have been perceived as a very stressful event for the animals, resulting in a strong behavioural response from the lambs regardless of their level of sociability.

6. Conclusions

In this study we have identified promising non-invasive indicators of negative arousal in lambs. The identification of behavioural and vocal indicators of this emotional dimension may allow the reduction of animal exposure to stressful situations, although the identification of indicators of positive arousal is also necessary and currently under-researched. Although there is partial evidence for the use of maximum eye temperature as a physiological indicator of negative arousal, more research is needed to clarify its actual potential for use.

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Supplementary materials

Figure S1. Experimenter hide (top left) and the isolation pen (top right)

Tables for model 1.

Table S2. Linear Mixed-Effects Model results (referred to behaviour PC1)

	Chisq	Df	Pr(>Chisq)
Phase of the test	96.00	1	< 0.0001***
Social score	0.19	1	0.66
Lamb size	0.18	1	0.68
Phase of the test *social score	0.94	1	0.33
Phase of the test*lamb size	3.95	1	0.04698*
Social score*lamb size	0.11	1	0.74

*Significance codes: * = 0.05; ** = 0.01; *** = 0.001*

Table S3. Model estimates (referred to behaviour PC1)

Phase of the test	Lamb size	emmean	SE	df	lower.CL	upper.CL
Full isolation	Big	1.44	0.46	28.1	0.51	2.38
Partial isolation	Big	-1.26	0.46	28.1	-2.19	-0.32
Full isolation	Small	1.99	0.46	28.9	1.05	2.93
Partial isolation	Small	-2.14	0.46	28.9	-3.08	-1.20

Table S4. Post hoc test (referred to PC1)

Contrast	estimate	SE	df	t.ratio	p.value
Full big – partial big	2.70	0.51	17.0	5.35	0.00
Full big – full small	-0.55	0.65	28.3	-0.85	0.83
Full big – partial small	3.58	0.65	28.7	5.50	<.0001
Partial big – full small	-3.25	0.65	28.7	-4.99	0.00
Partial big – partial small	0.88	0.65	28.3	1.362	0.53
Full small – partial small	4.13	0.53	17.0	7.84	<.0001

Table S5. Linear Mixed-Effects Model results results (referred to behaviour PC2)

	Chisq	Df	Pr(>Chisq)
Phase of the test	2.19	1	0.14
Social score	0.11	1	0.74
Lamb size	2.79	1	0.09
Phase of the test *social score	0.32	1	0.57
Phase of the test*lamb size	0.20	1	0.65
Social score*lamb size	0.02	1	0.89

*Significance codes: * = 0.05; ** = 0.01; *** = 0.001*

Tables for model 2.

Table S5. Linear Mixed-Effects Model results results (referred to acoustic PC1)

	Chisq	Df	Pr(>Chisq)
Phase of the test	4.47	1	0.03455*
Lamb size	1.50	1	0.22
Phase of the test*lamb size	0.72	1	0.40

*Significance codes: * = 0.05; ** = 0.01; *** = 0.001*

Table S6. Models estimates (referred to acoustic PC1)

Phase of the test	emmean	SE	df	lower.CL	upper.CL
Full isolation	0.01	0.66	4.02	-1.84	1.85
Partial isolation	-0.39	0.68	4.51	-2.21	1.42

Table S7. Linear Mixed-Effects Model results results (referred to acoustic PC3)

	Chisq	Df	Pr(>Chisq)
Phase of the test	10.41	1	0.001252**
Lamb size	0.54	1	0.46
Phase of the test*lamb size	4.20	1	0.040494*

*Significance codes: * = 0.05; ** = 0.01; *** = 0.001*

Table S8. Models estimate (referred to acoustic PC3)

Phase of the test	Lamb size	emmean	SE	df	lower.CL	upper.CL
Full isolation	Big	-0.70	0.43	4.30	-1.86	0.46
Partial isolation	Big	-0.98	0.48	6.73	-2.13	0.17
Full isolation	Small	-0.13	0.42	3.88	-1.30	1.05
Partial isolation	Small	-1.28	0.50	7.89	-2.43	-0.12

Table S9. Post hoc test (referred to acoustic PC3)

Contrast	estimate	SE	df	t.ratio	p.value
Full big – partial big	0.28	0.29	147.89	0.96	0.77
Full big – full small	-0.57	0.60	4.09	-0.96	0.78
Full big – partial small	0.57	0.66	5.97	0.87	0.82
Partial big – full small	-0.85	0.64	5.22	-1.33	0.58
Partial big – partial small	0.29	0.70	7.30	0.42	0.97
Full small – partial small	1.14	0.31	147.92	3.69	0.00

Table S10. Linear Mixed-Effects Model results results (referred to acoustic PC4)

	Chisq	Df	Pr(>Chisq)
Phase of the test	16.70	1	< 0.0001***
Lamb size	0.15	1	0.70
Phase of the test*lamb size	0.01	1	0.93

*Significance codes: * = 0.05; ** = 0.01; *** = 0.001*

Table S11. Model estimates (referred to acoustic PC4)

Phase of the test	emmean	SE	df	lower.CL	upper.CL
Full isolation	-0.55	0.30	4.05	-1.38	0.29
Partial isolation	0.08	0.33	5.63	-0.73	0.90

Tables for model 3.

Table S12. Linear Mixed-Effects Model results results

	Chisq	Df	Pr(>Chisq)
Phase of the test	20.84	1	< 0.0001***
Delta from phase start	4.40	1	0.0360*
Social score	2.66	1	0.10
Lamb size	18.32	1	< 0.0001***
Phase of the test * delta from phase start	0.33	1	0.56
Phase of the test *social score	0.01	1	0.94
Phase of the test*lamb size	26.35	1	< 0.0001***
Social score*lamb size	0.69	1	0.40

*Significance codes: * = 0.05; ** = 0.01; *** = 0.001*

Table S13. Model estimates

Phase of the test	Lamb size	emmean	SE	df	lower.CL	upper.CL
Full isolation	Big	30.9	0.50	17.4	29.8	31.9
Partial isolation	Big	30.9	0.50	16.8	29.9	32.0
Full isolation	Small	28.7	0.50	17.7	27.6	29.7
Partial isolation	Small	27.3	0.50	17.3	26.2	28.3

Table S14. Post hoc test

Contrast	estimate	SE	df	t.ratio	p.value
Full big – partial big	-0.050	0.20	758.5	-0.25	0.99
Full big – full small	2.21	0.71	17.5	3.11	0.03
Full big – partial small	3.61	0.71	17.4	5.09	0.00
Partial big – full small	2.26	0.71	17.3	3.19	0.02
Partial big – partial small	3.66	0.71	16.9	5.20	0.00
Full small – partial small	1.40	0.21	760.0	6.70	<.0001

Tables for model 4.

Table S15. Linear Mixed-Effects Model results results

	Chisq	Df	Pr(>Chisq)
Behavioural score	28.41	1	< 0.0001***
Social score	0.43	1	0.51
Lamb size	0.04	1	0.84
Behavioural score*social score	0.21	1	0.65
Behavioural score*lamb size	15.17	1	< 0.0001***
Social score*lamb size	1.42	1	0.23

*Significance codes: * = 0.05; ** = 0.01; *** = 0.001*

Table S16. Model estimates

Lamb size	Behavioural score	SE	df	lower.CL	upper.CL
Big	-0.00	0.00	463	-0.00	0.00
Small	0.01	0.00	754	0.01	0.01

Table S17. Contrast of the model

Contrast	estimate	SE	df	t.ratio	p.value
Big – small	-0.01	0.00	580	-3.85	0.00

Tables for model 5.

Table S18. Linear Mixed-Effects Model results results (referred to acoustic PC1)

	Chisq	Df	Pr(>Chisq)
Behavioral score	5.70	1	0.017011*
Lamb size	7.63	1	0.005736**
CR	0.45	1	0.50
Behavioral score*lamb size	0.03	1	0.86
Behavioral score*CR	4.20	1	0.040409*
Lamb size*CR	0.07	1	0.78

*Significance codes: * = 0.05; ** = 0.01; *** = 0.001*

Table S19. Model estimates (referred to acoustic PC1)

CR	Behavioural score	SE	df	lower.CL	upper.CL
-0.05076	-0.31	0.26	23.3	-0.85	0.22
0.00998	0.28	0.09	145.3	0.10	0.46
0.07071	0.87	0.35	27.8	0.15	1.59

Table S20. Linear Mixed-Effects Model results results (referred to acoustic PC3)

	Chisq	Df	Pr(>Chisq)
Behavioral score	0.36	1	0.55
Lamb size	0.73	1	0.39
CR	0.87	1	0.35
Behavioral score*lamb size	0.48	1	0.49
Behavioral score*CR	5.55	1	0.01847*
Lamb size*CR	0.43	1	0.51

*Significance codes: * = 0.05; ** = 0.01; *** = 0.001*

Table S21. model estimates (referred to acoustic PC3)

CR	Behavioural score	SE	df	lower.CL	upper.CL
-0.05076	-0.39	0.21	22.7	-0.83	0.05
0.00998	0.16	0.10	131.2	-0.02	0.35
0.07071	0.72	0.29	23.6	0.11	1.33

Table S22. Linear Mixed-Effects Model results results (referred to acoustic PC4)

	Chisq	Df	Pr(>Chisq)
Behavioral score	13.77	1	0.0002069***
Lamb size	0.09	1	0.76
CR	1.93	1	0.17
Behavioral score*lamb size	4.12	1	0.0424860*
Behavioral score*CR	1.36	1	0.24
Lamb size*CR	0.06	1	0.81

*Significance codes: * = 0.05; ** = 0.01; *** = 0.001*

Table S23. model estimates (referred to acoustic PC4)

Lamb size	Behavioural score	SE	df	lower.CL	upper.CL
big	-0.19	0.09	96.9	-0.36	-0.01
small	-0.47	0.12	171.6	-0.71	-0.23

6. General conclusions

The overall aim of this thesis was to improve human understanding of emotions in small ruminants, with a focus on the study of vocalisations as an indirect indicator of emotional states.

The importance of studying emotions lies in the potential for humans to reduce the exposure of animals to negative and/or stressful situations. At the same time, it is important to maximise the possibility that animals can also, if not predominantly, experience positive emotions, in order to guarantee that they can live a life that is worth living (Mellor, 2016). Recent advances in animal welfare science on positive experiences in the lives of animals, or "positive welfare", meet the expectations of consumers and citizens who are increasingly aware and concerned with these issues (Mellor, 2016; Blokhuis et al., 2003).

In farmed animals, the quality of the human-animal relationship is deeply connected with the emotional state of the animals (Waiblinger et al., 2006). A fundamental prerequisite for establishing a positive human-animal relationship is the ability of humans to understand the signals emitted by animals and to be aware of how them react to human behaviour (Nawroth, 2017). For this reason, the first paper presented in this thesis is a review ("Humans and Goats: Improving Knowledge for a Better Relationship") addressing the human-goat relationship, focusing on human-goat communication, the factors that influence this relationship, the methods we can use to measure it and how it can be improved. In this paper, we reviewed the intentional communication signals between goats and humans and we found that acoustic communication between the two species has deserved very little attention so far, and that scientific studies have predominantly focused on acoustic signals emitted by humans, while those emitted by goats towards humans have received scarce attention. The review highlighted the importance of early, frequent and positive interactions with goats to improve HAR. Some socio-demographic variables, such as gender, influence behaviour towards animals, with women appearing to be more attentive to the emotional states of goats, whereas the study of other individual characteristics, such as attitudes, deserves further investigation. The review also highlighted the existence of a significant number of validated measures for the assessment of the quality of the human-goat relationship, albeit recorded by unfamiliar assessors and not designed for feasible self-assessment by farmers or owners. Finally, in the paper we suggested that increasing farmers' awareness that goats are able to express emotions may help them to perceive these animals as individuals and to develop more positive attitudes and a sense of commitment and responsibility towards them.

To support farmers in the interpretation of the vocalizations emitted by the goats and to provide them with an early warning system about their goats' emotional states, in the VOCAPRA Project we aimed at developing a continuous, non-invasive monitoring system (WASN) by means of vocalizations analysis. The use of Precision Livestock Farming (PLF) technologies, such as acoustic sensors, made it possible to record bleats emitted by goats from four farms over the course of a year. In the first experimental work of this thesis, we present the initial results of this work, which required a preliminary elaboration of all the sounds recorded on the farms in order to clean the information by filtering the "noise" (e.g., sounds emitted by the milking machine or by other machinery, people's voices, etc.). Subsequent manual labelling of these acoustic signals, organised according to the emotional states of the animals (by attributing emotions-generating contexts to the bleats), allowed the creation of a database, whose analysis revealed statistically significant differences among the different contexts. The study also showed the potential use of the database to train and test artificial intelligence algorithms for automatic recognition of goats' emotional states, to be implemented in a smartphone application as a useful tool to help farmers interpret goats' vocalisations. The database of bleats associated with emission contexts has been made public (https://zenodo.org/records/7530401) to be used by the scientific community for further research into the interpretation of goat vocalisations, but also by people and goat farmers to learn how to interpret goats' emotional states through their vocal expressions. In fact, farmers' understanding of animals' needs and emotional states is fundamental to establish and maintain a good HAR, and animal welfare may be posed at risk when farmers become over-reliant on PLF technologies, losing this capacity (Tuyttens et al., 2022). For these reasons, in the second experimental part of this thesis we also tried to improve our knowledge on humans' ability to understand goats' vocalisations and to identify their underlying emotions, depending on previous experience with goats, level of empathy towards goats, and other individual characteristics that may affect animals' emotion recognition.

Our results show a good humans' ability to recognise the emotional dimension of valence in goat vocalisations, as evidenced by the qualitative description of these sounds by the participants in our study, which was consistent with the emission contexts. Although the respondents were able to correctly classify the bleats above the chance level three out of four emission contexts, this ability was higher for negative contexts. This was probably due to the more important information conveyed by goats in negative situations of urgency than in positive situations. The ability of humans to correctly attribute goats' bleats to contexts of emission was found to be influenced by the experience with these animals, with people with frequent direct contacts with goats showing higher correct classification rates. These results suggest the importance of the presence of experienced people to ensure good welfare, not only at farm level, but in any context where human-goat interactions may occur. Having a high empathy level with goats seemed to improve the discrimination of bleats depending on the context of emissions, even if the role of empathy, attitudes and their underlying factors have to be further investigated. Finally, the human ability to interpret goats' vocalizations did not seem to be affected by other individual characteristics such as having children, gender and university education.

In the last experiment, we moved to a different species, i.e. sheep, and tried to use of vocalisations in combination with other behavioural and physiological indicators to interpret the emotional state of lambs. In fact, while some promising indicators of emotions have been identified in goats (Briefer et al., 2015; Baciadonna et al., 2016), only few studies have been carried out so far in sheep, especially on the vocal correlates of emotion (Sèbe et al., 2012; Papadaki et al., 2021). Therefore, we aimed to fill this gap in knowledge by identifying potential vocal indicators of negative arousal in lambs. To this end, we subjected the lambs to a two-phases isolation test (partial *vs* full) designed to induce negative emotions, but characterised by different intensities of emotional arousal. Behavioural and vocal responses of individuals to isolation were assessed, along with maximum eye temperature, measured using thermography. We identified vocal correlates of negative arousal in lambs, that produce more vocalizations per time unit and with higher frequencies (energy quartiles, formants, fundamental frequency) and Wiener entropy. Moreover, these vocal indicators were found to increase with bodily activation of the individuals as the arousal increased. In contrast, the results referred to physiological indicators were found to be inconsistent, but the potential to use thermography as a method to assess animals' emotions (Arfuso et al., 2022; Comin et al., 2023) deserve further investigation. Finally, we found an effect of the size of the lambs on their behavioural response to isolation, that needs further investigation. The individuals' sociability did not affect the lambs' response to isolation, contrary to our *a priori* hypothesis. This could be explained by the fact that the separation from conspecifics may have been perceived as a very stressful emotional experience for animals belonging to a social and gregarious species such as sheep, leading to a strong behavioural response, independently from their level of sociability.

In conclusion, this thesis shed light on how small ruminants express their emotions, particularly through vocal production. To achieve this, the emotional content of vocalisations has been interpreted through traditional acoustic analysis, namely the extraction of acoustic features and subsequent application of statistical models, but also through direct human understanding of acoustic signals and the use of automated systems. The practical implications of this work lie in the improvement of the human-animal relationship and the level of welfare resulting from a better understanding of the acoustic signals emitted by sheep and goats. The use of PLF technologies has shown interesting potential for this purpose (Neethirajan et al., 2021), although not without some limitations. Relying solely on technology could lead to a reduction in interactions with animals, with negative consequences for the human-animal relationship. In addition, these tools may experience technical problems or provide inaccurate predictions (Tuyttens et al., 2022). The study on the interpretation of emotions in small ruminants still reveals some gaps in our knowledge that could be addressed by future research. If there is evidence for non-invasive indicators of emotion in goats, little is known about sheep, particularly on the emotional content of their bleats. In fact, there are no studies that have identified potential correlates of valence or that have explored the human ability to decode the emotional information of their vocalizations.

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