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Effects of pain on the facial expressions of goat kids

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ABSTRACT

Pain assessment is essential in laboratory and farm animals. Grimace scales have been used for this purpose since 2010. The aim of the present study was to investigate how pain (due to disease presence) affects the main facial expressions of goat kids. For this purpose, 60 goat kids aged 1 to 20 days were included in the study. An experienced veterinarian graded the animals' pain based on his experience (pain 0, 1, and 2), and two photographs (frontal and lateral) were taken. The following measurements were recorded: Height and width of the palpebral fissure, mouth angle, angles of the nose in profile and front. Pain level affected the height (0.83 and 1.29 cm, pain level 0 and 2 respectively, $p < 0.001$) and width of the palpebral fissure (1.85 and 2.35 cm, pain level 0 and 2 respectively, $p < 0.001$), which increased at pain level 2. Thus, narrowing of the eye was not observed when pain level increased. The angle of the mouth increased at pain level 2 (39.2 and 41.0 degrees, pain level 0 and 2 respectively, $p = 0.013$), and the frontal angle of the nose decreased at pain level 2 (93.5 and 85.0 degrees, pain level 0 and 2 respectively, $p = 0.009$).

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Pain evaluation; goat kids; grimace; facial

Introduction

Pain assessment in animals is a relevant topic for researchers, animal scientists and veterinarians in a daily basic frequency. European Directive 2010/63/EU is very clear regarding the assessment of pain in scientific procedures involving animals. It requires procedures to be classified as 'non-recovery', 'mild', 'moderate', and 'severe'. United States Department of Agriculture also establishes a classification of pain level into four categories (B, C, D and E, Animal Welfare Act 2024). Pain assessment is critical on farms because there is a direct correlation between pain, stress, and poor performance (Chulayo and Muchenje 2015). Veterinarians need to assess animal pain in order to prescribe treatment and evaluate the progress of recovery.

The 3Rs (Replacement, Reduction, Refinement) concept proposed by Russell and Burch (1959) includes pain assessment as a key factor to improve refinement. The use of grimace scales was initially developed to humans, where emotions are reflected in facial expressions. This scale is useful for nonverbal human patients (Hicks et al. 2001). The pain-face relationship was first used by Langford et al. (2010) to assess pain in mice. These authors correlated orbital constriction, nasal bulge, cheek bulge, ear position, and whisker changes with the degree of pain.

After 2010, grimace scales were developed for other laboratory animals such as rats and rabbits. More recently, grimace scales have been developed for farm animals such as ewes (McLennan et al. 2016; Hager et al. 2017), lambs (Guesgen et al. 2016), cattle (Muller et al. 2019), pigs (Vullo et al. 2020), or piglets (Viscardi et al. 2017), but little information is available for goats or their kids.

Regarding goats, Lou (2020) conducted a study on the application of the grimace scale in goat kids during disbudding. The study concluded that the grimace scale is a valid and reliable tool for assessing acute pain in goat kids undergoing disbudding. Additionally, the research found that the use of local anesthesia can significantly reduce the pain associated with the procedure.

In a more recent study, Weeder et al. (2023) described an experiment aimed at determining the optimal dose of amphotericin B to induce transient lameness in meat goats for research purposes. The authors developed a facial grimace scale for goats to evaluate their pain responses. According to the paper, the optimal dose of amphotericin B was found to be 5 mg/0.25 mL, resulting in the most severe and consistent lameness among the goats. The study also introduced a goat grimace scale based on five facial features that can be utilized to assess pain in goats (ear position, nostril shape and dilation, orbital tightening, and cheek tightening).

Recently, Hussein and Al-Nakshabendy (2023) explored the use of facial expressions and infrared thermography to measure positive emotions in goats. The authors stroked the goats' bodies in three areas (forehead, neck, and withers) and observed their facial grimace scale, ear postures, and surface temperatures. The study revealed that stroking induced

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significant changes in most facial units, ear positions, and eye and nasal temperatures, indicating a positive emotional valence in goats. The paper concludes that facial expressions and peripheral temperatures are vital indicators of positive emotions in goats.

Due to a prevailing lack of knowledge regarding the utilization of facial expressions as pain indicators at the farm level, the primary objective of the present study was to comprehensively evaluate the impact of pain on the facial expressions of goat kids without inducing any deliberate pain stimuli under farm conditions. In light of the limited understanding in this area, the study sought to bridge the gap by examining how pain manifests in the natural environment of the farm setting and assessing the corresponding facial expressions exhibited by goat kids under these conditions. The investigation aimed to contribute valuable insights into the recognition and interpretation of facial expressions as potential indicators of pain in goat kids without the influence of deliberate pain induction procedures.

Material and methods

Ethical issues

All procedures incorporated into the current study fall outside the scope of Directive 2010/63/EU due to the specific definition of a procedure outlined in the directive. According to the directive, a 'procedure' encompasses any use, whether invasive or non-invasive, of an animal for experimental, scientific, or educational purposes, with known or unknown outcomes. Moreover, it includes activities that may cause the animal a level of pain, suffering, distress, or lasting harm equivalent to, or greater than, that caused by the introduction of a needle in accordance with good veterinary practice.

In the context of our study, it is crucial to emphasize that the procedures undertaken did not induce pain in the animals. The only stress experienced by the animals was associated with the gentle handling required during the experimental protocols. This distinction is paramount in ensuring compliance with ethical standards and animal welfare guidelines. By explicitly highlighting that the procedures did not cause pain but only mild stress associated with gentle handling, we underscore the ethical and humane nature of our approach. This clarification further solidifies the ethical foundation of the study, aligning with the principles of minimizing harm and ensuring the welfare of the animals involved.

Animals and study location

In this research endeavour, a total of sixty Majorera goat kids, comprising 30 males and 30 females, were deliberately chosen for the study. These animals, artificially reared to ensure controlled conditions, spanned an age range of 1 to 20 days, a critical period in their early developmental stages. The meticulous selection of subjects was conducted from a diverse pool, originating from three distinct livestock farms situated in southeastern Gran Canaria, Canary Islands, Spain. The geographical coordinates of the study locations are recorded as 27.8724N latitude and −15.5012W longitude, providing a precise reference point for the study's contextualization within the specific environmental conditions of this region. The intentional inclusion of both genders and the age diversity within the cohort aim to capture a comprehensive snapshot of developmental nuances and potential gender-specific variations in the parameters under investigation. This meticulous approach to subject selection and detailed geographical specification ensures the robustness and generalizability of the findings within the specific context of the Majorera goat population in southeastern Gran Canaria.

Disease evaluation

An experienced veterinarian classified the animals into three categories (adapting the classification from Zentrich et al. 2023) of pain based on their clinical signs: Pain 0 – no pain (no evidence of disease), Pain 1 – moderate pain (some signs of disease but able to stand), and Pain 2 – severe pain (severe signs of disease and difficulty standing) (see Table 1). The most frequent signs were nasal discharge and respiratory distress due to pneumonia, lameness, joint swelling, and perianal fecal contamination from diarrhea.

Imagen capture and facial action quantification

In the course of this study, the animals were gently restrained for a brief duration, during which two photographs were captured utilizing a digital camera, specifically the Nikon D3100 (Tokyo, Japan). The photographic documentation was executed at a standardized distance of 60 cm from each animal, ensuring consistency in image acquisition. Notably, two images were captured for each subject – a frontal view and a profile view from the right side.

The photographic sessions transpired within the confines of the farm's yards. This strategic decision was made to minimize stress on the animals, as relocating them to an alternative location could potentially induce distress. By conducting the photographic documentation within familiar surroundings, we aimed to maintain a stable and comfortable environment for the subjects, thereby safeguarding the integrity of the collected data.

Table 1. Clinical score.

Parameter	Clinical signs	Score
Vocalization	None	0
	Slightly muffled voices	1
	Muffled voices	2
Activity	Sleeping and resting	0
	Frequent change of position	1
	Restless, directionless walking	2
Food/water or milk intake	Normal,	0
	Reduced	1
	Inappetence	2
General appearance	Sniffing and looking for straw, hay, or wáter,	0
	playing with neighbourns	1
	Downcast, turning head to side	2
	Apathetic	
Pain	No evidence of disease	0
	Innespessific clinical signs but able to stand	1
	Severe signs of disease: pneumonia (discharge and respiratory distress), lameness, joint swelling or perianal fecal contamination from diarrhea	2

Adapted from Zentrich et al. (2023).

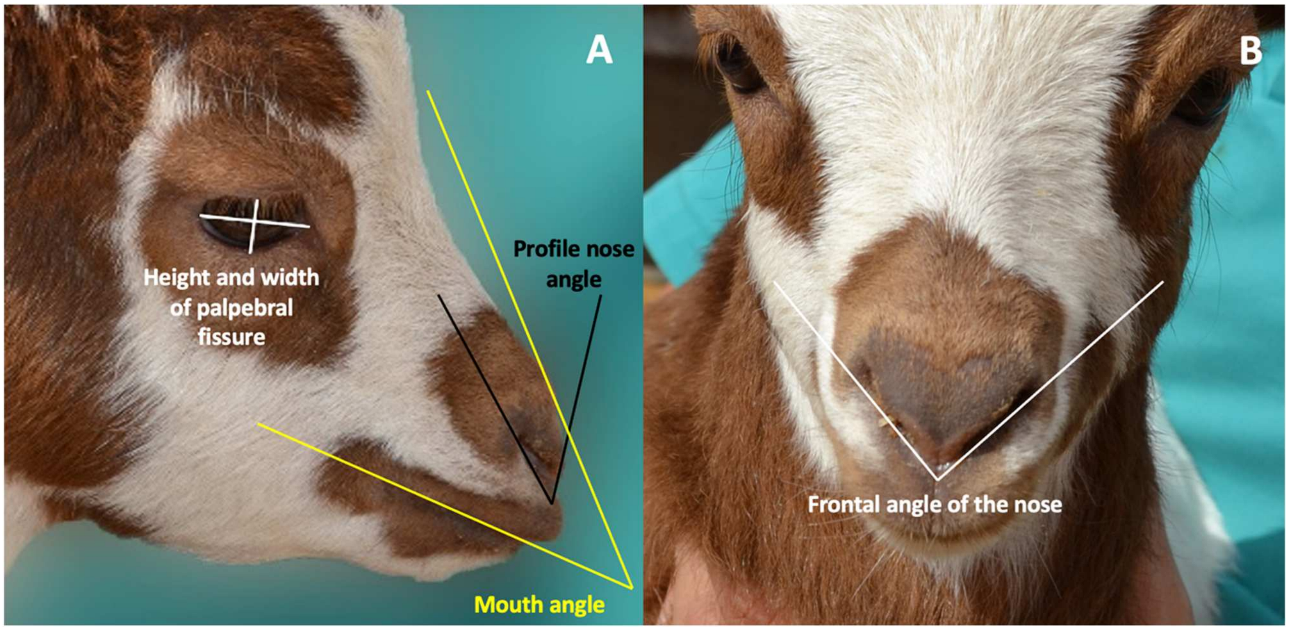

Figure 1. Determination of height and width of palpebral fissure, angle of the mouth, and frontal and in profile angles of the nose. A, height of palpebral fissure, straight line from the centre of the lower eyelid to the centre of the upper eyelid, intersecting the midpoint of the pupil. Width of palpebral fissure, straight line from the lateral to the medial corner of the right eye, crossing the middle of the pupil. Profile nose angle, one axis was placed over the right nostril, and the other axis was aligned to touch the most rostral point of the nose. The connecting vertex was then situated at the most ventral point of the nose. Mouth angle, one axis parallel to the oral commissure, while the other axis was drawn sagittally from the frontal bone at the level of the supraorbital foramina, tangentially touching the tip of the nose. The connecting vertex was positioned in front of the upper lip. B, frontal angle of the nose, two axes were strategically positioned over each nostril, with the connecting vertex situated at the convergence point of the nostrils.

In the current investigation, our focus centered on the quantification of three distinct facial expressions: eye opening, nose angles, and mouth angle. To precisely assess these expressions, a total of five measurements were undertaken, targeting specific features such as the height and width of the palpebral fissure, the angle of the mouth, and the angles of the nose in both profile and frontal views (refer to Figure 1).

For the measurements of the palpebral fissure, Adobe Photoshop (Adobe Systems Incorporated, U.S.A., V. CS4, 11.0) was employed to determine both the height and width. The height measurement involved drawing a straight line from the centre of the lower eyelid to the centre of the upper eyelid, intersecting the midpoint of the pupil. Meanwhile, the width measurement was conducted by drawing a straight line from the lateral to the medial corner of the right eye, crossing the middle of the pupil (see Figure 1).

To measure the frontal angle of the nose, two axes (represented by yellow and blue squares in Figure 1) were strategically positioned over each nostril, with the connecting vertex (depicted by the red square) situated at the convergence point of the nostrils. In the profile picture, one axis (yellow square) was placed over the right nostril, and the other axis (blue square) was aligned to touch the most rostral point of the nose. The connecting vertex (red square) was then situated at the most ventral point of the nose.

The measurement of the mouth angle involved the placement of one axis parallel to the oral commissure, while the other axis was drawn sagittally from the frontal bone at the level of the supraorbital foramina (blue square in Figure 1), tangentially touching the tip of the nose. The connecting vertex (red square) was positioned in front of the upper lip to ensure accurate measurement and representation of the mouth angle. These measurements were facilitated using the online software RULER, a tool developed by the Polytechnic University of Valencia, Spain (available at https://www.ergonautas.upv.es/herramientas/ruler/ruler.php).

Statistical analysis

Analysis was performed using RStudio version 1.1 (RStudio Inc, Massachusetts, U.S.A.). Statistical significance was set at $P \leq 0.05$. Normality was checked using the Shapiro–Wilk test. The height and width of palpebral fissure, mouth angles, and profile angle of the nose were normally distributed ($P = 0.145$, 0.078, 0.559, and 0.258, respectively). The frontal angle of the nose was not normally distributed ($P = 0.001$).

The effect of pain level on height and width of palpebral fissure, mouth angle, and profile nose angle was assessed using the one-way ANOVA. Differences between means were tested using the Tukey HSD test. The effect of pain level on the frontal angle of the nose was evaluated using the Kruskal–Wallis test. Differences between mean values were tested using pairwise comparison with the Wilcoxon rank sum test.

Results and discussion

Tables 2 and 3 present comprehensive descriptive statistics delineating the observed measurements based on their adherence to either normal or non-normal distribution patterns. Notably, in the context of livestock species research (Guesgen

Table 2. Descriptive statistics of frontal angle of the nose in degrees.

	Minimum	1st Quartile	Median	3rd Quartile	Maximum
Frontal angle of the nose	49.00	83.75	89.00	94.25	102.00

Table 3. Descriptive statistics for height and width of palpebral fissure, angle of the mouth and in profile angle of the nose.

	Minimum	Mean	Standard deviation	Maximum
Height of palpebral fissure (cm)	0.62	1.09	0.30	1.83
Width of palpebral fissure (cm)	1.37	2.13	0.41	3.53
Angle of the mouth (degrees)	31.00	39.15	4.12	50.00
Angle of the nose in profile (degrees)	26.00	48.48	10.99	80.00

et al. 2016; Viscardi et al. 2017), the predominant methodology has involved the application of grimace classification, as established in mouse pain assessment models (Langford et al. 2010). In this paradigm, expressions of pain are categorized into degrees – ranging from absent to moderate to severe – rather than being quantified in conventional units such as centimetres or angular degrees.

Regrettably, this divergence in the approach to pain measurement has rendered cross-study comparisons challenging. The absence of a standardized metric, such as a common unit of measurement, hinders the juxtaposition of findings across various manuscripts. Consequently, this methodological incongruity precludes a meaningful comparison with the broader body of literature in the field.

The Table 2 shows the minimum, 1st quartile, median, 3rd quartile and maximun for the Frontal angle of the nose (49, 83.75, 89, 94.25 and 102 degree, respectively).

Significant variations in pain levels were discerned in the dimensions of the palpebral fissure, mouth, and frontal nasal angles among goat kids, as illustrated in Tables 4 and 5. The phenomenon of eye narrowing, a hallmark feature across various Grimace scales (Guesgen et al. 2016; Viscardi et al. 2017; Lou 2020; Weeder et al. 2023), is consistently described. Eye narrowing is characterized by tension in the ocular region, leading to a reduction in palpebral fissure width and eventual eye closure.

Contrary to findings in mice (Langford et al. 2010), rats (Sotocinal et al. 2011), seals (MacRae et al. 2018), and even goats (Lou 2020; Weeder et al. 2023), Table 4 unveil a noteworthy divergence. With an escalation in pain levels from 0 to 2 (Table 4), there was a significant increase in both palpebral fissure height and width. This contrasts with the observed eye closure in other species under similar circumstances. The apparent controversy in these outcomes could be attributed to two primary factors. First, there is the proposition, as posited by Mogil et al. (2020), that species exhibit differential grimacing responses to acute noxious stimuli. Second, the temporal aspect of grimacing is crucial, with chemical/inflammatory or postsurgical procedures reported to manifest over varying timeframes – ranging from minutes to hours or even after 24 h following the stimuli (Mogil et al. 2020).

Table 4. Least square means of mouth angle and side nose angle according to pain classification.

Measures	Pain 0	Pain 1	Pain 2	SD	*P*
Height of palpebral fissure (cm)	0.83[a]	1.15[b]	1.29[b]	0.30	0.001
Width of palpebral fissure (cm)	1.85[a]	2.19[b]	2.35[b]	0.41	0.001
Angle of the mouth (degrees)	39.20[ab]	37.25[a]	41.00[b]	4.12	0.013
Angle of the nose in profile (degrees)	52.50	46.80	46.15	10.99	0.133

SD, standard deviation. Different letters in the same row means least squares means statistical differences according *P* value.

Table 5. Kruskal–Wallis analysis and post hoc Wilcoxon rank for Frontal angle of the nose.

	Pain 0	Pain 1	Pain 2	*P*
Frontal angle of the nose (degrees)	93.50[a]	87.00[ab]	85.00[b]	0.009

Different letters in the same row means statistical differences according *P* value.

It is pertinent to note that the magnitude of pain in our study was contingent upon farm-related illnesses, and the temporal dynamics of grimacing were not explicitly controlled. Nonetheless, this approach aligns with a pragmatic perspective, aimed at providing veterinarians with a genuine and applicable tool for on-farm pain assessment.

The angle of the mouth (Table 4) was significantly higher in pain level 2 than in pain level 1, but both (pain levels 1 and 2) showed no differences from pain level 0. The mouth angle is not present in the grimace scales of mice (Langford et al. 2010) or rats (Sotocinal et al. 2011), but was used by (Guesgen et al. 2016) in lambs. The latter authors described that lambs in pain show a flatter lip line than lambs not in pain, and that are consistent with what is indicated in Table 4 for mouth angle. It is important to note that the increase in the angle of the mouth is small and likely difficult for veterinarians or goat farmers to observe on the farm.

The level of pain significantly decreased the frontal angle of the nose ($P = 0.009$). Pain level 2 was significantly different from pain level 0. Similar results were observed by (Guesgen et al. 2016) who described the reduction in the frontal angle of the nose as a pointed nose.

The grimace scales of sheep, mice, rats, rabbits, and horses show similarities that support the idea that emotions are associated with similar facial expressions in all mammalian species, as suggested by Williams (2002) and Dalla Costa et al. (2014). However, it is important to note that the same individuals were involved in the creation of all grimace scales, which may have resulted in some overlap due to their prior knowledge of changes in facial features. Furthermore, the differences in facial expressions between the grimace scales could be due to variations in facial size, composition, and musculature between goat kids, lambs, mice, rats, and rabbits. In addition, it is possible that different types of pain (e.g. disease-related, thermal, chemical, or mechanical pain) elicit slightly different facial expressions, although this requires further investigation.

Conclusion

The present study has shown that the facial expressions of goat kids change as a function of pain intensity on farm condition.

The question is whether these changes in facial expression can be easily detected by veterinarians or goat keepers. Another limitation of the present study was the persistence of grimacing after noxious stimuli triggered by the pathological process. However, the strategy of measuring facial expressions opens a new way to evaluate pain in farm animals.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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