

## Behavioural and physiological response to routine husbandry procedures in Wallachian sheep

Lucia Kotianová, Tereza Lakomá, Zdeňka Vacuškova, Dominik Vacuška, Vladimír Večerek, Eva Voslářová

University of Veterinary Sciences Brno, Faculty of Veterinary Hygiene and Ecology, Department of Animal Protection and Welfare and Veterinary Public Health, Brno, Czech Republic

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### Abstract

The social nature of sheep makes isolation a major factor affecting not only the welfare of the isolated individual but potentially the entire flock. This study assessed the impact of visual contact with the stressor (wool shearing and hoof trimming) during routine husbandry procedures using infrared thermography (IRT) and behavioural analysis. Sheep were divided into two groups with visual contact during the procedures (VC group) and without visual contact during the procedures (NC group). For each animal, eye temperature and behavioural assessments were conducted before and during the procedure period. The VC group exhibited significantly more active stress-related behaviours, such as increased active time and an absence of lying down, compared to the NC group ( $P < 0.05$ ). In contrast, the NC group maintained higher levels of inactivity and exhibited some instances of lying down. Infrared thermography measurements revealed a significant increase in right eye temperature in both groups by the end of the procedures compared to pre-procedure levels ( $P < 0.05$ ). Notably, the VC group demonstrated a more pronounced increase in ocular temperature than the NC group during the finishing phase ( $P < 0.05$ ), indicating heightened stress responses. These findings suggest that visual contact with conspecifics during husbandry procedures does not mitigate stress but may instead amplify arousal and stress-related responses. This study underscores the importance of considering social factors in animal welfare practices and supports the use of IRT as a reliable, non-invasive tool for stress assessment in sheep.

*Ovine, infrared thermography, wool shearing, hoof trimming*

Social isolation induces fear and stress in social animals such as sheep, profoundly impacting behaviour and welfare of the individuals. Stress levels increase significantly in isolated sheep that are unable to maintain visual contact with other flock members, compared to those that can see their conspecifics (Kannan et al. 2021). Wool shearing is a routine management practice in sheep husbandry, as domestic sheep do not naturally shed their wool. However, common husbandry procedures, particularly wool shearing, can elicit both endocrine and metabolic responses in sheep (Carcangiu et al. 2008). During shearing, sheep are generally exposed to short-term social and tactile isolation, although visual contact may still be maintained (Fidan et al. 2009). Tactile isolation has been reported to increase plasma hormone and metabolites indicative of stress in small ruminants subsequently impacting their immune function (Degabriele and Fell 2001). Moreover, sheep can be affected not only by procedures they undergo but also by witnessing the distress of their conspecifics. The extent of this impact may depend on their capacity for empathy (Edgar et al. 2012).

Nowadays, significant attention is given to non-invasive approaches for evaluating acute stress in animals. Among the widely used non-invasive methods for stress assessment, salivary cortisol measurement has been frequently applied in farm animals such as horses, cattle, goats, and pigs (Němečková et al. 2022; Popelková et al. 2022;

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#### Address for correspondence:

prof. Ing. Eva Voslářová, Ph.D.  
Department of Animal Protection and Welfare and Veterinary Public Health  
Faculty of Veterinary Hygiene and Ecology  
University of Veterinary Sciences Brno  
Palackého tř. 1946/1, Brno 612 42, Czech Republic

E-mail: [voslarovae@vfu.cz](mailto:voslarovae@vfu.cz)  
<http://actavet.vfu.cz/>

Hostovská et al. 2024). Andanson et al. (2020) have demonstrated that collecting saliva samples does not trigger cortisol release, unlike venipuncture, which does in animals that are not habituated to the procedure. Other assessment methodologies include hair sampling, heart rate monitoring, and infrared thermography (IRT) (Sharma et al. 2019; Joy et al. 2022). Infrared thermography emerges as a non-invasive, contactless alternative for stress assessment, particularly useful for sheep unaccustomed to human interaction (Bartolomé et al. 2019). Infrared thermography can detect changes in peripheral blood flow and heat loss, making it a potentially valuable tool for measuring stress in mammals (Kadlecová et al. 2024). Recent studies have shown that temperature changes around the eyelid and the caruncula lacrimalis, areas with dense capillary networks innervated by the sympathetic nervous system, respond to alterations in blood flow.

Behavioural responses are fundamental mechanisms that help animals adapt to environmental changes. However, the complexity of measuring stress using behavioural analysis could be challenging to what could be considered reliable. Behavioural manifestations indicative of stress in sheep may include escape attempts, baulks, increased vocalization or position of the head (Hemsworth et al. 2011; Hemsworth et al. 2019). Gougoulis et al. (2010) emphasize the need to consider the lack of expression in sheep when identifying distress signs, as they may react to stressors with immobility instead of active responses. Additionally, facial expressions can serve as indicators of pain in farmed sheep (McLennan et al. 2016).

This study aimed to evaluate the impact of visual contact during routine husbandry practices, such as wool shearing and hoof trimming, in Wallachian sheep. The effects of visual contact on the sheep's emotional status have not yet been systematically assessed using both behavioural and physiological measures such as IRT.

## Materials and Methods

### Animals and procedures

The subjects of the study were 20 adult Wallachian sheep (*Ovis aries*) of both sexes, randomly selected from a flock situated in the Moravian Wallachia region of the Czech Republic. The selected individuals were randomly divided into two groups: a visual contact group (VC group,  $n = 10$ ), which maintained visual contact with the rest of the flock during routine husbandry procedures, specifically wool shearing and hoof trimming, and a non-visual contact group (NC group,  $n = 10$ ), which did not have visual contact during the same procedures. Prior to the procedures, the sheep were placed in two distinct semi-indoor pens. The VC group had direct visual access to both the shearer and the sheep being sheared, while the NC group only had acoustic and olfactory contact during the shearing process, without any visual contact. Both groups were separated by a fixed pen and had *ad libitum* access to hay and fresh water. Straw was used as bedding in both pens. The study was conducted in accordance with the relevant legislation of the corresponding country.

### Data collection and statistical analysis

Behavioural responses were recorded using two video cameras (Niceboy VEGA PRO; GoPro HERO 10) both equipped with built-in microphones. Data were collected in March 2023, during the usual shearing period, for 45 min before the procedures (pre-procedure period) and for 45 min during the procedures for the VC group as well as NC group separately. During the pre-procedure period, all 20 sheep were housed together in a single pen. For behaviour coding, the recorded videos (with audio) were analyzed using Observer XT software (v.16, Noldus Information Technology, the Netherlands) to determine the duration of various behavioural responses (Table 1). To assess inter-observer reliability, scores from the first scorer were validated with that of a second scorer, who coded four videos from the VC and four from the NC group. Cohen's kappa indicated agreement between the coders across all coded behaviours (all  $\kappa > 0.97$ ,  $P < 0.05$ , all  $\rho > 0.97$ ,  $P < 0.05$ ). A continuous sampling technique was used to analyse collected behavioural data.

Additionally, IRT was utilized for non-invasive, non-contact measurement of physiological changes, namely to observe changes in eye temperature in response to the wool shearing and hoof trimming in both groups. Infrared thermography images (thermograms) were taken using a portable thermal imaging camera, testo 890-2 (Testo SE & Co. KGaA, Titisee-Neustadt, Germany). The image parameters were set to  $640 \times 480$  pixels, with a sensitivity of  $< 0.04$  °C and a temperature range of  $-30$  to  $100$  °C. The emissivity was determined to be 0.98, based on the generally accepted emissivity of an animal's body (Byrne et al. 2019). For this analysis, six thermograms of the right eye area of each sheep ( $n = 20$ ) were captured at a  $90^\circ$  angle from a distance of 1 metre. The camera was handheld to accommodate possible movements of the sheep's head. All images were taken by

Table 1. Definitions of behavioural measures that were included in the behavioural analysis.

Behaviour	Definition
Time inactive	The sheep is standing still with no movement of the legs.
Locomotion	At least two legs of the sheep are moving.
Lying down	The sheep is resting on the ground, with legs either extended or folded beneath its body.
Grooming	The sheep is scratching using objects, horns, limbs, or teeth.
Food intake	The sheep is sniffing, extracting, and chewing feed, followed by the absence of subsequent rumination.
Rumination	The act of rumination that does not immediately follow feed intake.
Fixed stare	The sheep maintains fixed gaze in one direction for over 2 seconds.

the same operator in a location shielded from direct sunlight. During data collection, the ambient temperature was 5 °C, and the relative humidity was 65%. Following a habituation period of 5 min, two thermograms of the right eye of each sheep were obtained, and a photograph of the corresponding ear tag was also taken for identification purposes. Subsequently, two thermograms for each sheep were recorded at the start of the procedures while each sheep was individually held by the shearer. Two additional thermograms were captured immediately after the wool shearing and hoof trimming were completed, with the sheep still in the position held by the shearer. Each sheared sheep was seated on its rump, with its hind legs folded beneath. The shearer used their legs and body to support and stabilize the sheep, minimizing stress. The sheep was adjusted as necessary to enable the shearer to reach all parts of the body, with the head tilted slightly backward. Once the procedures were finished, the sheep was returned to the pen. Thermographic data were compared between the groups during the procedures and for each group, also data obtained in the pen (pre-procedure period) were compared with data obtained at the beginning and at the end of the procedures to observe the potential changes in eye temperature in response to the routine husbandry procedures. The area of the right eye is considered reliable for the measurement of body temperature in sheep with a high correlation (Dos Reis and White 2022). For the analysis of thermograms, IRSoft software (Testo SE & Co. KGaA, Titisee-Neustadt, Germany) was used.

Data were analyzed using the statistical package Unistat 5.6 for MS Excel (Unistat Ltd., London, England). The normality of the data was assessed using Shapiro-Wilk test. ANOVA was used to compare the means of two groups and determine if the difference in means was significant. A value of  $P < 0.05$  was determined as significant in statistical tests. For additional statistical analysis, R and RStudio were utilized.

## Results

The results of behavioural analysis are given in Table 2. A significant difference ( $P < 0.05$ ) was observed in both NC and VC groups when comparing the time spent being inactive between the pre-procedure and procedure periods. Moreover, the VC group spent significantly more time ( $P < 0.05$ ) being inactive than the NC group during the procedure. Regarding locomotion, sheep in both groups exhibited increased movement ( $P < 0.05$ ) during wool shearing and hoof trimming compared to the pre-procedure period. However,

Table 2. Percentage occurrence of selected behaviours before and during the monitored routine husbandry procedures.

Behaviour	Occurrence of selected behaviours (%) in the given period (45 min)		
	Pre-procedure period	During the procedure (NC group)	During the procedure (VC group)
Time inactive	43.12 <sup>a</sup>	67.87 <sup>b</sup>	85.07 <sup>c</sup>
Locomotion	2.86 <sup>b</sup>	8.43 <sup>a</sup>	6.22 <sup>a</sup>
Lying down	45.24 <sup>a</sup>	20.07 <sup>b</sup>	0.00 <sup>c</sup>
Grooming	1.88 <sup>a,b</sup>	4.33 <sup>a</sup>	0.39 <sup>b</sup>
Food intake	6.97 <sup>a</sup>	9.87 <sup>a</sup>	6.82 <sup>a</sup>
Rumination	29.53 <sup>b</sup>	57.07 <sup>a</sup>	21.84 <sup>b</sup>
Fixed stare	8.09 <sup>a</sup>	14.88 <sup>a</sup>	14.21 <sup>a</sup>

<sup>a,b,c</sup> Values within the same row followed by different superscripts are significantly different ( $P < 0.05$ )

NC group of sheep (n = 10) had no visual contact with the shearer and the sheep being sheared

VC group of sheep (n = 10) had a direct visual contact with both the shearer and the sheep being sheared

no significant difference ( $P > 0.05$ ) was found between the NC and VC groups. A significant decrease ( $P < 0.05$ ) was observed in the time spent lying down in both groups during the procedure compared to the pre-procedure period. Notably, the VC group did not lie down at all during the procedure, resulting in a significant difference ( $P < 0.05$ ) between the NC and VC groups. No significant difference ( $P > 0.05$ ) in grooming was detected between the pre-procedure and procedure periods. However, a difference between the groups was noted during the procedure. Food intake remained unchanged ( $P > 0.05$ ). For rumination, a significant difference ( $P < 0.05$ ) was found between the NC and VC groups during the procedure, with the NC group ruminating more. No significant difference ( $P > 0.05$ ) was observed in fixed staring behaviour.

The results of infrared thermography measurements are given in Table 3. The median temperature of the right eye did not differ ( $P > 0.05$ ) between the NC and VC groups in the pre-procedure period. At the beginning of the wool shearing phase (when the sheep were restrained by the shearer), the median surface temperature did not increase ( $P > 0.05$ ) compared to the pre-procedure period, and no significant difference ( $P > 0.05$ ) was found between the NC and VC groups. However, by the finishing phase of the procedure, the median ocular surface temperature had significantly increased in both NC and VC groups compared to the pre-procedure period and the beginning of the procedure (immediately after the sheep was placed in the shearing position). Significant differences ( $P < 0.05$ ) were also found between the NC and VC groups during the finishing phase of the procedure, with the VC group exhibiting higher median ocular surface temperatures than the NC group.

Table 3. Comparison of median ocular surface temperature (°C) before and during the monitored husbandry procedures.

Group	Median surface temperature of the right eye (°C)		
	Pre-procedure period	During the procedure (beginning)	During the procedure (finishing)
NC	37.40 <sup>a,x</sup>	37.60 <sup>a,x</sup>	38.10 <sup>b,x</sup>
VC	37.30 <sup>a,x</sup>	37.40 <sup>a,x</sup>	38.50 <sup>b,y</sup>

<sup>a,b</sup> Values within the same row followed by different superscripts are significantly different ( $P < 0.05$ )

<sup>x,y</sup> Values within the same column followed by different superscripts are significantly different ( $P < 0.05$ )

NC group of sheep (n = 10) had no visual contact with the shearer and the sheep being sheared

VC group of sheep (n = 10) had a direct visual contact with both the shearer and the sheep being sheared

## Discussion

Sheep are known to exhibit fear towards individuals they perceive as potential threats (Napolitano et al. 2011). It is well documented that handling and shearing cause increased cortisol levels in sheep, indicating a stress response (Stewart et al. 2007). The acute stress observed in our study is likely multifactorial, influenced not only by handling and separation but also by the direct sensory impact of shearing (Hawken et al. 2013). Wool shearing is a critical management practice with significant implications for sheep farming. Farmers commonly shear sheep to reduce the risk of external parasites and to enhance growth performance and adaptability to their environment (Piccione et al. 2008). However, the process of fleece removal has been identified as one of the most stressful experiences in a sheep's life, particularly due to the thermal stress it induces. The loss of insulation triggers physiological changes, including alterations in rectal temperature, respiratory and heart rates, red blood cell count, haematocrit, and haemoglobin levels. These variables serve as secondary stress indicators in shorn sheep and provide valuable insights into their physiological responses (Casella et al. 2016).

Acute stress in small ruminants can be assessed using various methods, with a growing emphasis on non-invasive techniques like salivary cortisol analysis, heart and respiratory

rates, or IRT (Dos Reis and White 2022; Popelková et al. 2022). A significant correlation has been found between maximum eye temperature and both salivary and plasma cortisol levels (Cook et al. 2001). This suggests that changes in eye temperature may be influenced by the activation of the hypothalamic-pituitary-adrenal (HPA) axis. However, Stewart et al. (2007) provided evidence that exogenous stimulation of the HPA axis alone does not lead to increases in eye temperature. Therefore, incorporating habituation procedures prior to sampling is crucial, especially in cognitive experiments, as sheep assess environmental events based on their predictability, which influences their emotional and behavioral responses (Greiveldinger et al. 2007).

Behavioural indicators of acute stress in sheep include reduced interest in food, rigidity, a fixed stare, and increased locomotion as animals attempt to escape the stressor (Al-Quarawi and Ali 2005). Parrott et al. (1994) confirmed that adult sheep exposed to psychological and physical stressors, such as isolation, transport simulation, and handling, exhibited elevated plasma concentrations of stress markers. In our study, visual contact with the shearer significantly influenced specific behavioural responses in Wallachian sheep, particularly standing and locomotion. During the shearing procedure, we observed significant increases in standing and locomotion, while lying behaviour decreased in both groups, regardless of visual contact with the shearer. This suggests that handling itself induced restlessness, in agreement with previous studies identifying both separation and shearing as major stressors in sheep husbandry (Parrott et al. 1994). Interestingly, food intake was not significantly impacted by the procedures in our study, which contrasts with findings from other authors (Romeyer and Bouissou 1992). However, rumination increased in the NC group compared to the pre-procedure period and the VC group during manipulation. This aligns with prior research reporting stable feeding behaviours in both shorn and unshorn sheep, likely due to consistent dietary conditions before and after shearing (Beatty et al. 2008; Naglaa et al. 2012). Nevertheless, our findings on rumination slightly diverge from previous studies that reported a decrease in rumination frequency in shorn sheep compared to unshorn ones (Badawy et al. 2008). This discrepancy may be due to breed-specific stress coping strategies or differences in experimental conditions, which should be explored further.

Sheep in the VC group exhibited the highest percentage of inactive standing behaviour, possibly driven by an increased motivation to escape. However, this behaviour could also be linked to the overall comfort of the sheep post-procedure or the presence of microinjuries. Some studies have reported that unshorn sheep stand less frequently than shorn sheep (Serdal et al. 2011), while others found no significant effect of shearing on standing behaviour (Naglaa et al. 2012). These differences suggest that standing behaviour immediately after shearing may vary based on individual responses and environmental factors. Additionally, breed affiliation has been noted as a factor influencing stress responses, with different breeds exhibiting varying reactions to stressors (Cannas et al. 2018). For example, studies on the Sarda breed confirmed breed-specific differences in stress responses (Beatty et al. 2008). Wallachian sheep are known for their strong flocking instinct and their suitability for maintaining and restoring mountain pastures (Milerski and Konrad 2021).

In our study, we observed no significant difference in grooming behaviour between the pre-procedure and procedure periods. However, a statistically significant difference was noted during the procedure between the visual contact (VC) and non-contact (NC) groups. This finding aligns with other studies that have highlighted variations in grooming behaviour in shorn and unshorn ewes during wool shearing, indicating that grooming behaviour is an important welfare indicator during this process (Naglaa et al. 2012). The increase in grooming observed in the NC group may be attributed to reduced stress levels associated with the lack of visual contact with the shearer. Conversely, the VC group's lower grooming

levels could reflect heightened stress due to the shearer's presence, reinforcing the idea that visual contact influences stress responses in sheep. Other authors have also reported that shearing induces various skin stimuli, leading to heightened grooming behaviours immediately after the process (Hart and Pryor 2004).

While thermal imaging shows promise for diagnosing acute stress, factors such as subject distance, humidity, and ambient temperature must be considered (Arfuso et al. 2022). Breed differences should be considered when assessing stress responses via thermography. A study on the Sarda breed using digital thermography found significant individual variations in ocular surface temperature responses to acute stress (Milerski et al. 2021). Although their sample size was small, the findings emphasize the importance of breed-specific considerations in thermographic stress assessment. Studies report inconsistent findings regarding temperature changes in animals under acute stress (Oka et al. 2001; Edgar et al. 2013). While stress generally leads to an increase in temperature, some studies document an initial rapid decrease due to peripheral vasoconstriction, followed by a temperature rise due to increased tissue metabolism triggered by glucocorticoid release. It has been argued that acute stress induces peripheral vasoconstriction, which initially decreases surface temperature. Later, an increase in temperature is observed due to increased tissue metabolism, a response triggered by glucocorticoid release (Oka et al. 2001).

The reasons behind the variations in eye temperature response to stress are not fully understood. However, differences in data collection protocols, such as the timing of thermal recordings, may contribute to inconsistencies across studies (Byrne et al. 2019). Our findings suggest that sheep with visual contact with the shearer exhibited higher levels of behavioural stress indicators and experienced a more rapid increase in eye temperature during the procedure compared to those without visual contact. This aligns with prior research that collected behavioural and IRT data 5- and 60-min post shearing (Schmidt et al. 2013).

These findings highlight the importance of considering social factors when designing husbandry practices to minimise stress. Both behavioural assessments and IRT proved to be viable, non-invasive methods for evaluating acute stress in sheep. Future research should focus on the time required for temperature recovery in different sheep breeds to refine stress management strategies and improve overall welfare in animal husbandry.

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