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Assessment of acute stress in alpacas (*Vicugna pacos*): A pilot study using salivary cortisol and infrared thermography

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Abstract

Alpacas are an increasingly popular hobby species in Europe, characterized by specific requirements and high sensitivity to stress-related factors. Therefore, it is essential to identify which situations or factors trigger stress responses and to apply appropriate, minimally invasive sampling methods for this purpose. A viable approach involves saliva sampling using specialized collection kits, which require minimal animal restraint and handling or the use of thermographic imaging to monitor changes in body surface temperature. In both cases, data and sample collection are non-invasive, without causing pain or compromising physical integrity of the animal. No significant increase in salivary cortisol concentration ($P > 0.05$) was observed in eight alpacas before and after veterinary examination. This result may be influenced by the fact that the animals were not habituated to saliva sampling, and the initial collection itself may have induced a stress response, leading to cortisol release even prior to the veterinary procedure. Consequently, the veterinary intervention did not result in a further increase in cortisol levels. The mean salivary cortisol concentrations before and after examination were 3,822.57 ± 698.32 pg/ml and 4,064.60 ± 1,217.55 pg/ml, respectively, with no significant difference ($P > 0.05$). The finding that capture alone acts as a significant stressor for alpacas is further supported by a significant increase ($P < 0.05$) in the surface temperature of the inner corner of the eye. The temperature measured remotely (36.35 ± 0.91 °C) increased significantly following capture during the veterinary examination (37.56 ± 1.36 °C). Our findings support the potential use of infrared thermography as a sensitive, non-invasive stress indicator in alpacas.

Welfare, thermal camera, surface temperature, handling

Alpacas belong to the South American camelids (Goñalons 2008), and according to current research, their domestication occurred approximately 6,000–7,000 years ago in the central Andes (Goñalons 2008; Fan et al. 2020). These areas are characterized by specific climatic conditions, including high daytime temperatures followed by steep nocturnal drops (McMullen 2008), as well as relatively nutrient-poor grazing lands. Alpacas are well adapted to these conditions through distinct morphological and physiological traits (Tynes 2010). Alpacas have been imported to Europe since the 1990s, and they have recently gained popularity as companion animals (Castilla-Castaño et al. 2021). Failure to meet the species-specific biological and husbandry needs of alpacas, especially when combined with environmental conditions that differ markedly from their native habitat, may compromise their welfare. Welfare deficiencies have been described even in commonly farmed animal species in the Czech Republic (Švestková et al. 2024a,b). Given that alpacas are a relatively recent addition to Central European livestock, these risks may be even more pronounced.

Alpacas are herd animals (Pollard and Littlejohn 1995) and, like other camelids, exhibit marked sensitivity to stress (Whitehead 2013). Physiological challenges such as pregnancy and lactation, illness, or additional stressors require increased energy expenditure for compensation. In alpacas, these conditions often lead to reduced feed

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intake, and prolonged exposure to stress may result in weight loss and the development of hepatic lipidosis (Van Saun 2006) or the formation of ulcerations (Whitehead 2013).

Welfare in alpacas can be assessed using various methods. Behavioural assessment includes monitoring changes such as escape attempts, stamping, kicking, spitting, or a tense facial expression characterized by pinned-back ears and wide-open eyelids, often accompanied by vocalization (Waiblinger et al. 2020; Kapustka and Garbiec 2022). Traditionally, stress in animals has been evaluated using invasive methods that require disruption of bodily integrity, such as blood sampling via venipuncture. More recently, non-invasive techniques have been introduced, which are more compatible with maintaining high welfare standards – a factor particularly important for stress-sensitive species such as alpacas. Stress is a physiological mechanism that enables adaptation to environmental changes (Möstl and Palme 2002), during which catecholamines and glucocorticoids are released (Marketon and Glaser 2008), and these substances can be measured in various sample types (Sheriff et al. 2011). However, some samples are unsuitable for stress assessment because the sampling procedure itself may induce significant stress, thereby distorting results and compromising the individual's welfare. For this reason, non-invasive sampling techniques are preferable (Cook 2012).

In addition to cortisol measurement in blood (Anderson et al. 1999), faecal cortisol analysis has been applied in alpacas (Arias et al. 2013). Among non-invasive methods suitable for evaluating acute stress, salivary hormone measurement has proven effective (Fell et al. 1985), and saliva can also be used to analyse other biochemical indicators (Hostovská et al. 2024). Saliva sampling has been used, for instance, to assess stress load during different restraint techniques employed during alpaca shearing (Wittek et al. 2017; Prágai and Kovács 2020). Another non-invasive technique is infrared thermography based on the measurement of surface temperature, an indicator that changes in response to stress. By capturing and detecting infrared radiation, this method allows for remote, non-contact measurement of the animal's surface temperature using a thermal camera. This technique has already been successfully applied in various domestic, companion, and wild animals for the evaluation of acute stress responses (Abdel-Hamid and Dawod 2020; Travain and Valsecchi 2021).

The aim of this study was to assess changes in salivary cortisol concentration and the surface temperature of the inner corner of the eye in alpacas exposed to acute stress during handling and veterinary examination.

Materials and Methods

Animals

The alpaca herd from which samples were collected consisted of eight individuals aged between 9 months and 16 years. The sex ratio was balanced, with four females and four males. All animals were housed in a pasture-based enclosure with free access to a shelter bedded with straw and provided with meadow hay and water *ad libitum*. Preventive health checks were conducted regularly, twice a year. The farm on which the herd was kept hosts regular public events and school excursions. The animals were accustomed to routine interactions with visitors and to daily care provided by the keepers. No handling of the animals beyond routine care was performed prior to sample collection.

Data collection

The animals were captured and restrained by trained caretakers during the morning hours. Saliva samples were collected during restraint, both before and after veterinary examination. The captures took place within the enclosure, in the presence of other herd members; visual contact between the animals was maintained throughout the procedure. The veterinary examination included a general health assessment, administration of vitamin A (Bioveta, Ivanovice na Hané, Czech Republic), and rectal collection of a faecal sample.

Saliva was collected using the Salivette® collection kit (Sarstedt, Nümbrecht, Germany). The collection involved inserting a cotton swab held with forceps into the animal's mouth, where it was chewed for 60–90 s. In the laboratory, the Salivette® collection tubes were centrifuged at 3,341 × *g* for 15 min at 21 °C. The resulting saliva samples were transferred into Eppendorf tubes and stored at −80 °C until analysis. Salivary cortisol concentrations were measured using a commercial Cortisol Express ELISA Kit (Cayman Chemical, Frederick, USA). The detection range of the kit was 39.1–5,000 pg/ml. Absorbance was measured using a colorimetric plate reader at a wavelength of 420 nm, and results were calculated using the spreadsheet tool provided by the kit manufacturer (Cayman Chemical).

Surface temperature was recorded using a Testo 890-2 thermal camera (Testo SE & Co. KGaA, Titisee-Neustadt, Germany) with emissivity set to 0.97. Images were captured at a right angle to the alpacas' heads from a distance of approximately 0.5 m, focusing on the eye region to obtain sharp images. For each animal, three images were taken prior to handling, during which the person with the thermal camera spent some time in the enclosure allowing the animals to habituate to their presence and subsequently ignore them, and three images were captured during the veterinary examination (restraint and examination).

Surface temperature values were extracted from the inner corner of the eye using IRSoft software (Testo SE & Co. KGaA). For each individual (male 1–4 and female 1–4) and each phase (before handling, during examination), the mean temperature was calculated from the three images. Blurred images were excluded from the analysis.

Statistical analysis

Statistical analyses were performed using UNISTAT for Excel version 6.5 (Unistat Ltd., London, UK). Salivary cortisol concentrations in alpacas before and after the examination, as well as surface temperature values before handling and during veterinary examination, were compared using paired Student's *t*-test. Probability values of < 0.05 were considered significant.

Results

Table 1 presents the salivary cortisol concentrations in alpacas before and after veterinary examination. The sample collected from female 2 prior to examination was contaminated and therefore excluded from further analysis. The mean cortisol concentration before examination was 3,990.72 (± 547.86) in males and 3,598.36 (± 938.50) in females. The mean cortisol concentration after examination was 4,513.93 (± 1,409.60) in males and 3,615.27 (± 871.36) in females. No significant differences were found between pre- and post-examination values, or between males and females ($P > 0.05$).

Table 1. Salivary cortisol concentrations (pg/ml) in alpacas before and after veterinary examination.

	Salivary cortisol concentration (pg/ml)	
Alpacas n = 8	Before examination	After examination
Male 1	4,537.77	6,590.99
Male 2	3,616.85	3,456.25
Male 3	4,376.58	4,069.09
Male 4	3,431.69	3,939.39
Female 1	2,910.83	3,931.38
Female 2	-	4,677.70
Female 3	3,216.69	2,703.58
Female 4	4,667.57	3,148.40
Mean ± SD	3,822.57 ± 698.32[a]	4,064.60 ± 1,217.55[a]

[a] Same superscripts in the same row indicate no significant difference ($P > 0.05$)

Table 2 shows the surface temperature values of the inner corner of the eye in alpacas measured remotely before examination and subsequently after capture during veterinary examination. The mean temperature before examination was 36.9 ± 0.31 °C in males and 35.73 ± 0.90 °C in females. The mean temperature recorded during the examination was 38.52 ± 0.87 °C in males and 36.61 ± 1.06 °C in females. A significant increase ($P < 0.05$) in the surface temperature of the inner eye corner was observed in relation to handling and veterinary examination; however, no significant difference was found between males and females ($P > 0.05$).

Table 2. Surface temperature (°C) of the inner corner of the eye in alpacas before and during veterinary examination.

Alpacas n = 8	Surface temperature of the inner corner of the eye (°C) Before examination (measured remotely)	During examination (measured while animal was restrained)
Male 1	36.90	39.30
Male 2	37.40	37.30
Male 3	36.65	38.93
Male 4	36.95	38.53
Female 1	36.40	36.60
Female 2	34.40	36.65
Female 3	36.10	37.90
Female 4	36.00	35.30
Mean ± SD	36.35 ± 0.91[a]	37.56 ± 1.36[b]

[a,b] Different superscripts in the same row indicate a significant difference ($P < 0.05$)

Discussion

The saliva collection method has gained popularity in recent years due to its non-invasive nature and relatively simple and rapid laboratory processing, as well as the possibility to store saliva samples for extended periods at low temperatures (−80 °C). Consequently, this method is now widely used across various animal species and different contexts. Prágai and Kovács (2020) monitored changes in salivary cortisol concentrations before, during, and after shearing in alpacas. The values measured the day before shearing in alpacas housed overnight in stables ranged from 0.6 to 3.81 nmol/l (i.e., 217 to 1,381 pg/ml). The cortisol concentrations observed in our study prior to examination were higher than those reported in their study; however, as noted by Raggi et al. (1994), cortisol levels in alpacas are higher in the morning compared to the evening, indicating that the time of day may influence absolute values obtained from samples. Salivary cortisol concentrations measured 30 min before shearing in the study by Prágai and Kovács (2020), when the alpacas were already restrained, ranged from 0.88 to 54.94 nmol/l (i.e., 319 to 19,915 pg/ml), with most values falling below 6.32 nmol/l (i.e., 2,291 pg/ml). These baseline values are also lower than those found in our study.

Wittek et al. (2017) also investigated salivary cortisol concentrations during restraint of alpacas. In their study, alpacas were restrained for 15 min either standing, lying down, or lying on a table. An increase in cortisol concentration was evident in all groups at 20 and 40 min. In our study, the animals were restrained while standing, but the second sampling occurred approximately 10 min after the first, which may be an insufficient period to observe pronounced changes. According to Majchrzak et al. (2015), who studied the use of salivary cortisol in dromedaries, salivary cortisol is a suitable indicator particularly for short-term stress responses. The authors monitored stress levels during periods of varying riding frequency and outside the tourist season. Salivary cortisol values ranged from 600 to 2,500 pg/ml, with lower values observed during the tourist season when dromedaries were used for rides. Such comparisons can be very useful in assessing animal welfare in relation to their commercial use. These values are considerably lower than those found in our study, which may be attributed to species differences or to the fact that in the case of alpacas, capture from the herd preceded the examination.

Although saliva collection is considered a non-invasive method, the animals must first be captured and restrained, which may directly cause an increase in salivary cortisol concentrations. However, to date, no studies have reported the time course in which salivary cortisol elevation occurs in alpacas. For this reason, cortisol concentrations measured prior to veterinary examination may already reflect elevated stress levels due to restraint. Additionally, the animals experienced saliva collection for the first time in their lives. Stress induced by saliva sampling, however, can be mitigated by habituating animals to regular saliva collection, which involves only brief chewing of the cotton swab.

Our results suggest that the veterinary examination itself may not cause a further increase in salivary cortisol concentrations. To date, no studies have specifically monitored stress responses during veterinary examinations in alpacas. However, a study investigating the effects of various procedures in dairy cattle found significant differences in plasma cortisol levels between experimental and control groups during procedures such as artificial insemination, embryo transfer, natural mating, and hoof treatment. Similar studies would be highly valuable for alpacas to better understand which management procedures are the least stressful and how habituation may influence stress responses in these animals.

An increase in surface temperature during acute stress responses has been confirmed in various animal species; however, this method has not yet been applied to alpacas. The rise in body surface temperature results from the combined action of stress hormones, which cause vasodilation manifested as a transient increase in temperature in peripheral body regions (Cabezas et al. 2007; Herborn et al. 2015; Bartolomé et al. 2019). When using thermal imaging, it is necessary to identify hairless areas on the animal where skin temperature can be measured directly without interference from wool, fur, or feathers, so-called ‘thermal windows’ (Andrade 2015; Lowe et al. 2019). From this perspective, the inner corner of the eye in alpacas appears ideal because there is no insulating wool covering the skin, allowing heat to transfer to the surface. Another potentially suitable site could be the thermal windows in the axillary region, which serve to cool the body surface in camelids and represent relatively large areas of thinned skin on the abdomen, axilla, and inner thighs (Gerken 2010). However, these sites are difficult to access with a thermal camera and temperature measurements may be influenced by friction between skin surfaces. Even in the case of the inner eye corner, it is inappropriate to record animals that are tearing, soiled, or heated by sunlight; therefore, environmental conditions play a crucial role when using thermal imaging and must be considered (Jimoh and Ewuola 2018).

In addition to an overall increase in temperature, a short-term decrease followed by a subsequent rise – where the difference between the lowest and highest values can reach up to 3 °C – has sometimes been described (Bartolomé et al. 2019). This phenomenon was not observed in alpacas; however, the average temperature difference between before and after capture during veterinary examination was 1.21 °C. This transient vasoconstriction may last only a very short time and thus be difficult to detect, making the use of thermal cameras capable of video recording ideal for capturing such events. Narrowing of blood vessel lumens immediately following exposure to strong stressors has been reported, for example, in domestic rabbits (Blessing 2003).

Overall values alone cannot be used to infer the intensity of the stressor’s effect on the animal, as individual variability plays a significant role and temperature increases may differ between individuals. However, thermal images and their analysis can provide information on the duration of the animal’s stress response by indicating how long elevated body temperature persists, and whether habituation to a specific factor occurs, thus leading to a shortened recovery time to baseline values. For example, in rats, baseline temperature was reached only after 30 min following exposure to a stressor (Vianna and Carrive 2005). Nonetheless, prior experience with the situation likely plays a major role (Valera et al. 2012). Such assessments can be useful, for instance, in selecting the least stressful methods for necessary husbandry procedures, such as shearing, where the goal is to induce only short-term stress responses. The time required for return to baseline (i.e., the animal's resting state) also represents a valuable subject for further research. Handling or examination by a veterinarian is stressful for alpacas, as confirmed by our results, and this is supported by studies in other species such as horses (Yarnell et al. 2013), dogs (Travain et al. 2015), rabbits (Kadlecová et al. 2024), and goats (Bartolomé et al. 2019). Interestingly, visual contact with familiar conspecifics does not always have a calming effect; in some cases, observing other herd members undergoing handling or interventions may provoke a stress response in bystanders as well (Kotianová et al. 2025).

Other stressors investigated using thermal imaging in various species include temperature increases associated with emotional states (Chotard et al. 2018), heat stress (de Lima et al. 2013), and pain (Fleischmann et al. 2009). Additionally, thermal cameras appear to be a suitable tool for the early detection of certain diseases involving inflammatory responses like laminitis in horses (Rosenmeier et al. 2012). In animals sensitive to stress during handling, thermal imaging may be a more appropriate method for determining baseline values, as the data collector can maintain a distance of several metres from the animals.

In conclusion, saliva collection and measurement of salivary cortisol represent a non-invasive and validated method for assessing acute stress responses in various animal species, including alpacas. Elevated cortisol concentrations may already be detected during sampling immediately after capture due to necessary handling or the observation of conspecifics in stressful situations, especially in stress-sensitive species such as alpacas. For more reliable results, animals should be habituated to saliva sampling beforehand to prevent biasing the measured indices. Conversely, the use of thermal imaging does not require physical contact with the animal for temperature measurement, which can be particularly advantageous in field conditions. The inner corner of the eye, which is not covered by fleece, appears to be a suitable site for thermographic assessment, where an increase in surface body temperature was demonstrated during veterinary examination involving health evaluation, intramuscular vitamin supplementation, and collection of additional samples. The application of thermal imaging can aid in evaluating stress during husbandry procedures in alpacas, identifying particularly stressful and challenging factors for these animals, and exploring strategies to mitigate stress, thereby maintaining a high level of welfare in the care of these increasingly popular companion animals. However, to fully establish its utility, further validation is needed across larger and more diverse populations, as well as in varied management contexts. Future research should focus on standardizing protocols and exploring the integration of infrared thermography into routine veterinary procedures and animal welfare monitoring systems.

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