Bulgarian Journal of Veterinary Medicine, 2023 ONLINE FIRST
ISSN 1311-1477; DOI: 10.15547/bjvm.2022-0123

Original article

MEASURING POSITIVE EMOTIONS IN FREE-RANGE SHEEP USING PERIPHERAL TEMPERATURES, FACIAL ACTION UNITS AND EAR POSTURES

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Summary

Hussein, N. J. & A. A. Al-Naqshabendy, 2023. Measuring positive emotions in free-range sheep using peripheral temperatures, facial action units and ear postures. *Bulg. J. Vet. Med.* (online first).

The purpose of this study was to elucidate the effect of stroking ewes' body as a positive emotional stimulant on peripheral temperatures, facial action units and ear postures. Thirty-eight healthy ewes were included in the current study. The animals were stroked individually on neck, withers and forehead. Then the procedure was divided into three stages: pre-stroking, stroking and post-stroking with five minutes each. Peripheral temperatures were measured from eye, nose and ear using infrared thermal camera. Images were also captured to analyse facial units in studied sheep. For measuring head behaviours and ear postures, the ear change as a frequency per minute was measured at 30-second intervals for 15 respective minutes. During stroking, the temperature of eye, ear and nasal areas has significantly dropped (P<0.05), however it raised in eyes during the post-stroking period. Significant differences (P<0.05) were also found between pre-stroking and post-stroking phases in ear and nose temperatures. For facial units, significant differences (P<0.05) were found between pre-stroking, compared to stroking and post-stroking phases for all five facial units. In addition, the total facial action score was significantly (P<0.05) higher during the stroking and post-stroking phases, compared to the pre-stroking phase. Results obtained from ear positions showed that the time spent with ears in plane position had significantly decreased (P<0.05) in the stroking phase compared to pre- and post-stroking phases. In addition, ewes spent more time with their ears backward (P<0.05) during stroking in comparison to pre- and post-stroking phases. No significant differences were found in asymmetrical and forward ear postures. In comparison to the pre-stroking stage, the number of ear position change was not significantly increased during stroking, however, it increased considerably (P<0.05) in the post-stroking period. From this study, it was concluded that stroking ewes' body parts improved significantly the positive emotional state in healthy animals.

Key words: facial expressions, peripheral temperatures, positive emotions, sheep, stroking

INTRODUCTION

Animal welfare science is concerned with improving the living conditions of animals. In addition, it is concerned with improving nutrition, management, housing, slaughter, sanitation, health as well as the expression of normal behaviour (Machado & Silva, 2020). Many studies concentrate on negative states and on how to reduce or prevent them (McLennan *et al.*, 2016). Animal welfare is not only related to the negative states, such as disease and stress, but also to presence of positive experience (Green & Mellor, 2011; Mellor, 2015; Mattiello *et al.*, 2019).

Positive emotions can be induced by gentle interactions between animals and humans (Schmied *et al.*, 2008; Reefmann *et al.*, 2009; Coulon *et al.*, 2015). These interactions can occur through some senses like vision, olfaction, audition and physical or tactile stimuli (Waiblinger *et al.*, 2006). Previous research used body stroking of animals to induce positive emotions in cattle (Schmied *et al.*, 2008; Lange *et al.*, 2020) and sheep (Hussein, 2018). In order to assess welfare of animals using human-animal interaction, both physiological and behavioural indicators are used (Mendl *et al.*, 2010). The physiological indicators include measurements such as blood parameters, heart rate and body temperature, while behavioural measures can include head behaviours such as head stretching, ear positions and postures, and recently facial expressions (Mendl *et al.*, 2010; Descovich *et al.*, 2017).

Considering physiological indicators, the peripheral temperature was used. An increase in the body temperature for a short period is known as emotional fever (Proctor & Carder, 2015a). Rectal temperature is usually used to indicate core body temperature of animals (Burfeind *et al.*, 2010). However, its measurement can cause stress or injury to animals or humans (Zhen *et al.*, 2014). Therefore, a non-invasive method using infrared thermal cameras to measure surface temperature is more convenient (Kunkle *et al.*, 2004; Qu *et al.*, 2020). Several areas are used to measure surface temperature such as eye, ear and nose. However, eye temperature has yielded the most reliable results (Zheng *et al.*, 2022). The emissivity, which is the ability of an object to radiate infrared energy may affect peripheral temperature measurement (Tattersall, 2016). The emissivity of most animals is between 0.86 and 0.98 (Zheng *et al.*, 2022). Thus, the emissivity of thermal camera must be checked so as to avoid its effect on the measurement (Zheng *et al.*, 2022). The hypothalamic-pituitary axis is stimulated during negative and positive states of animals, which in turn leads to an increase in catecholamine and glucocorticoid thus causing more heat loss (Jansen *et al.*, 1995). The heat loss can be detected by a drop in surface or peripheral temperatures e.g. nasal and eye temperatures (Stewart *et al.*, 2005; Proctor & Carder, 2016; Hussein, 2018).

Ear postures, or the frequency of ear posture change, has now obtained a vital role in indicating negative emotional states in farm animals (Guesgen *et al.*, 2016). However, up to date, few studies have been undertaken to measure ear postures to indicate positive emotional states in animals (Proctor & Carder, 2014). In previous studies on ear postures in painful states, most of them indicated that animals spent more time with ears backward (Sotocinal *et al.*, 2011; Keating *et al.*, 2012; Dalla Costa *et al.*, 2014). In small ruminants like sheep, the changes in the number of ear postures, asymmetrical ear postures were increased when experiencing pain (Reefmann *et al.*, 2009); however, they were decreased during positive states such as feeding fresh hay (Reefmann *et al.*, 2009).

Facial expressions have been mainly used in negative states like stress and diseases in farm and laboratory animals (McLennan *et al.*, 2016). Little is known about face expressions during positive emotional states such as stroking animal's body. For the eye, nose and cheek regions, they have been well studied in human and animals (Proctor & Carder, 2015b); however, for positive emotional states in animals they have not been tested yet. Lip and jaw profile has been an indicator of positive welfare states in humans and some animals like chimpanzees and cats (Descovich *et al.*, 2017) but so far, little is known about facial expressions in sheep. Therefore, the main aim of this study was to investigate the effect of positive condition (stroking) on sheep peripheral temperatures of eye, ear and nose, as well as on ear postures and facial expressions of free-range Hamdani sheep.

MATERIALS AND METHODS

Study area and subjects

A study was undertaken in Karne village in north Zakho City, and Batifa District from September 2021 to March 2022. Thirty-eight healthy ewes (aged 3–4 years) were included in this research.

Ethics approval

The procedure of this study was ethically approved by the Animal Ethics Committee of the Faculty of Sciences, University of Zakho, Kurdistan Region – Iraq with approval code ARC011.

Stroking procedure

The animals were stroked individually on the neck, withers and forehead for five minutes. The animal guard or owner was stroking sheep to reduce the stress on them. The owner caught each animal individually and waited for five minutes to remove the stress on an animal. Then the procedure was divided into three stages namely pre-stroking, stroking and post-stroking, with five minutes for each stage to allow the researcher to make comparisons among the three stroking phases. In each phase, data were collected twice, corresponding to stopwatch minutes:seconds 0:00, 4:30 (pre-stroking); 5:00, 9:30 (stroking) and 10:00, 14:30 (post-stroking).

Data collection

Temperature data. Peripheral temperatures were collected from eye, nose and ear using infrared thermal camera (FLIR E4, FLIR Systems, OU, Estonia). Eighteen thermal images were captured for ear, nose and eye from each ewe from all stopwatches; a total of 684 temperature data were obtained. Pictures were captured from a distance of half to one meter as previously done by Proctor & Carder (2015a). The measured temperature is shown on the left top corner in the infrared image (Fig. 1).

Facial grimace scale. Images were taken from side and front of the head of each ewe for every stopwatch using a high resolution Sony camera (SONY, Cyber-Shot, DSC-H20, Japan). Two pictures were taken from each ewe in each stopwatch, i.e 12 pictures for each subject with total of 456 pictures. The pictures were then cropped to include head only and were scored five months after the procedure to exclude any bias when scoring face actions (Fig. 2). The following facial

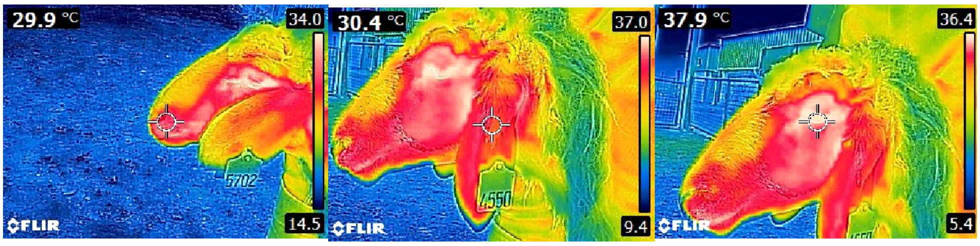

Fig. 1. Infrared thermal images showing the temperature of nose, ear and eye of ewes on the top-left corner of each image.

Fig. 2. Cropped images captured for ewes to detect face action units.

units were measured: orbital tightening, ear position, lip and jaw profile, cheek tightening, and nostril and philtrum shape (McLennan *et al.*, 2016).

Ear postures. For measuring head behaviours and ear postures, the ear change frequency per minute was measured at 30-second interval for 15 respective minutes, that is 30 ear postures were recorded for each animal with total of 1,140 ear postures for all ewes. The plane, forward, backward, and asymmetrical ear postures were measured (Boissy *et al.*, 2011).

Data analysis

All data were recorded in Microsoft Excel spreadsheet. The datasets were analysed using GenStat software (17^{th} edition, VSN International). Shapiro-Wilk normality test revealed that temperature data were parametric, whereas all other data including face action units, behaviour and ear postures were non-parametric. Thus, for temperature data, ANOVA one-way repeated measures was used with Fisher's unprotected LSD test for *post-hoc* comparisons. For non-parametric data, Kruskal-Wallis test was used following by Mann-Whitney U-test for *post hoc* comparisons. All tables and figures were arranged in Microsoft Excel spreadsheet and Past4 software (Paleontological Statistics, Version 4.09). The differences were considered to be significant at $P<0.05$.

RESULTS

Peripheral temperatures

Stroking ewes body had a significant effect on the eye ($P<0.01$), ear ($P<0.01$), and nasal ($P<0.05$) temperatures (Table 1). During stroking, eye, ear and nasal temperatures have significantly dropped, whereas they were raised in eyes after stroking. Significant differences ear and nose temperatures were also found be-

Table 1. Temperature (mean ± SEM; n=684) of eye, ear and nasal areas for the three stages of stroking

Body area	Phases			P value
	Before stroking	Stroking	After stroking	
Eye	36.5 ± 0.3a	35.3 ± 0.2b	35.9 ± 0.3ab	0.01
Ear	33.9 ± 0.5a	32.7 ± 0.5b	32.9 ± 0.6b	0.01
Nasal	34.0 ± 0.5a	32.1 ± 0.6b	33.0 ± 0.5b	0.05

Note: different letters in the same row indicate significant differences.

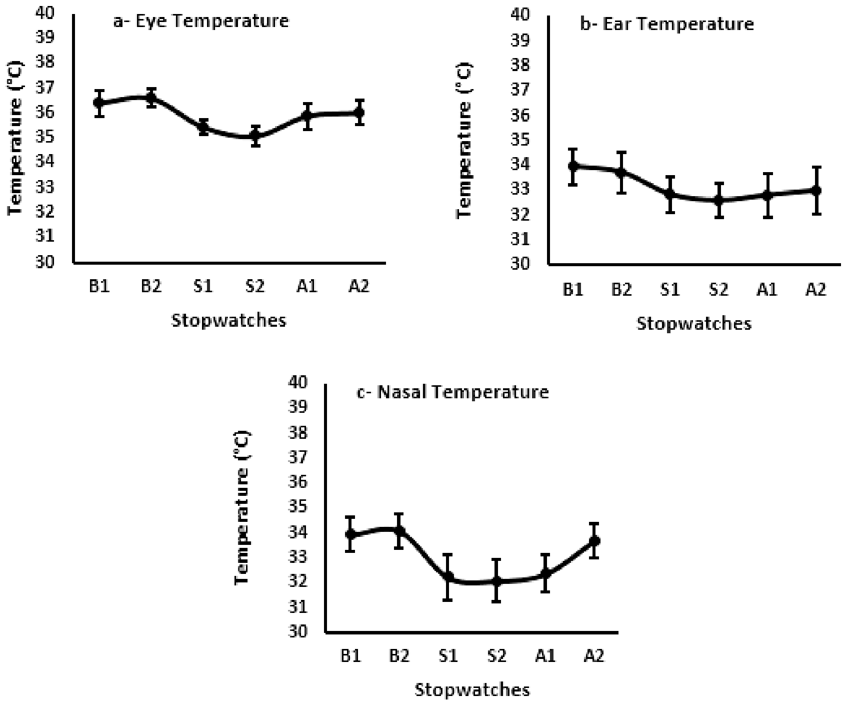

Fig. 3. Eye (a), ear (b) and nasal (c) peripheral temperatures throughout six different periods (mean ± SEM; n=684). B1 and B2 = before stroking; S1 and S2 = stroking; A1 and A2 = after stroking.

tween pre-stroking and post-stroking phases.

The mean eye, ear and nasal temperature over time for six stopwatches is shown on Fig. 3. The mean ± SEM (standard error of mean) eye temperatures were 36.4±0.5, 36.6±0.3, 35.5±0.3, 35.1±0.4, 35.9±0.5 and 36.0±0.4 C°.

Values for ear temperatures were 33.9±0.7, 33.7±0.8, 32.8±0.7, 32.6±0.7, 32.8±0.9 and 33.0±0.9 C° and for nasal temperatures: 33.9±0.6, 34.1±0.7, 32.2±0.9, 32.1±0.8, 32.4±0.7, and 33.7±0.7 C° for the stopwatches 0:00, 4:30, 5:00, 9:30, 10:00, and 14:30 respectively. The eye temperature has significantly ($P<0.05$)

dropped at 9:30 (min:sec) in comparison to 0:00 and 4:30 stopwatches. For ear temperatures, pre-stroking stopwatches (0:00 and 4:30) were significantly (P<0.05) different from all other stopwatches of stroking and post-stroking periods. For nasal temperature, pre-stroking value was significantly higher (P<0.05) than those during stroking (5:00 and 9:30) and post-stroking (only 10:00) periods (Fig. 3).

Facial grimace scale

Facial expressions of pre-stroking, stroking and post-stroking are shown on Fig. 4. Significant differences (P<0.05) were found between pre-stroking, compared to stroking and post-stroking phases. The

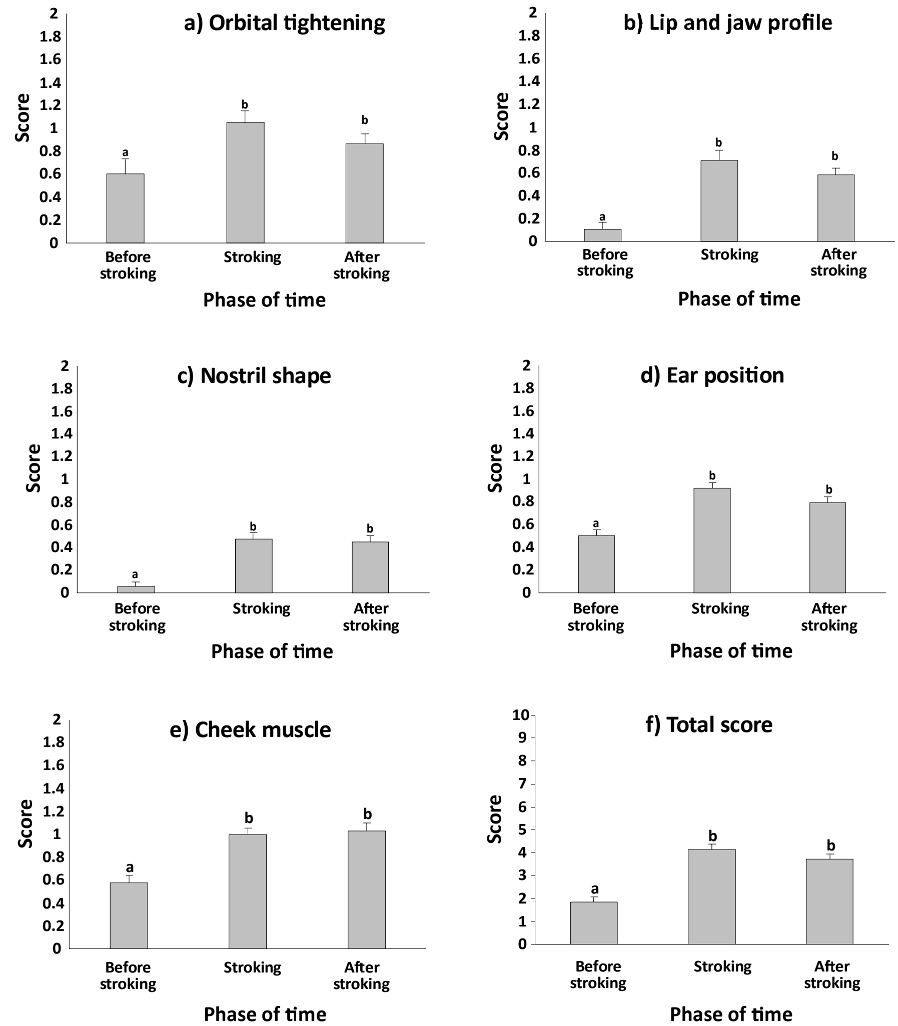

Fig. 4. Face scores (mean ± SEM; n=456) obtained from ewes before, during and after stroking: lip and jaw profile (a), orbital tightening (b), cheek (masseter) muscle (c), nostril and philtrum shape (d), ear position from side (e) and total face scores (f) for all five areas. Different letters on each figure indicate significant differences (P<0.05).

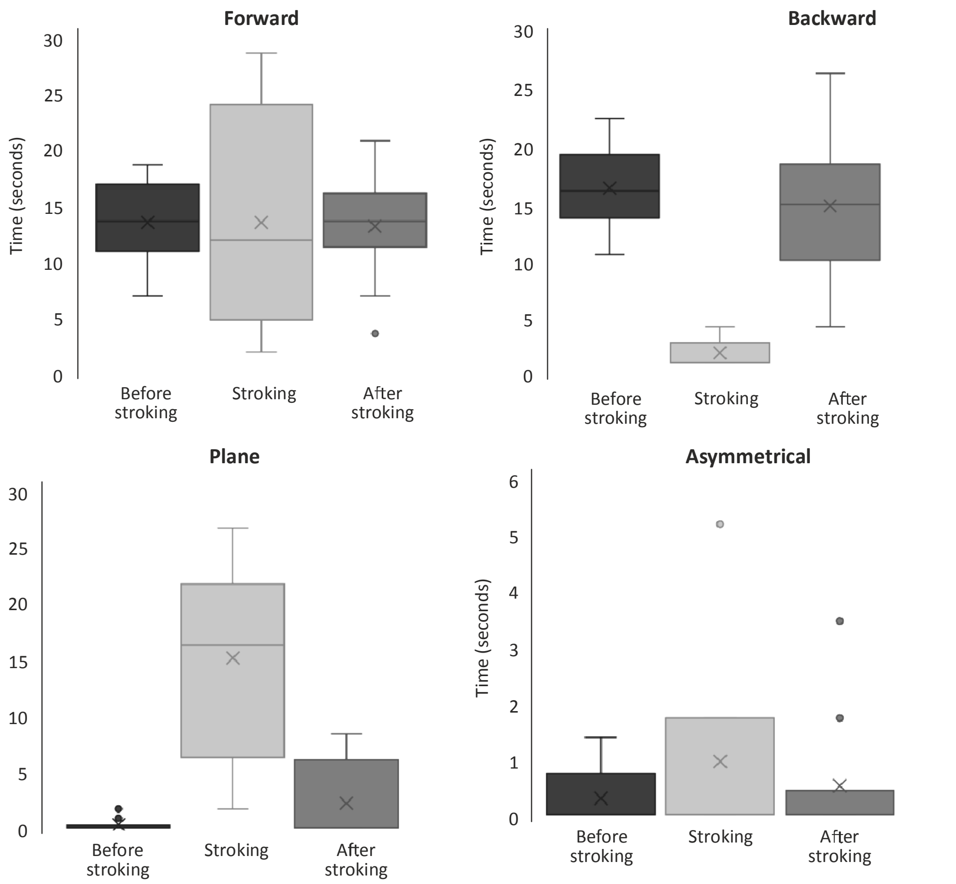

Fig. 5. Boxplots for ear postures in ewes before stroking, stroking and after stroking. White dots indicate moderate outliers and black dots – extreme outliers.

chin and jaw lines were more straightened during stroking and post-stroking (P<0.05) when compared to control (pre-stroking). For orbital tightening, ewes' eyes were more closed in stroking and post-stroking phases (P<0.05) in comparison to pre-stroking. The masseter muscle in cheek was more convex-shaped during stroking and the zygomatic arch increased more during the post-stroking period (P<0.05) vs the pre-stroking stage. The philtrum was shortened and narrowed and mimicked more V shape in stroking phases (P<0.05) when compared to pre-stroking (U shape). Ewes moved their ears more backward during stroking phases (P<0.05) compared to control (mostly plane) (Fig. 4 a-e). The total facial units for all measured areas were significantly higher (P<0.05) during stroking and after stroking stages in comparison to stroking phase (Fig. 4f).

Ear postures

The median of boxplots of ear postures experienced by ewes are illustrated on Fig. 5. Asymmetrical and forward ear postures were not significantly affected by stroking. Ear in plane position was sig-

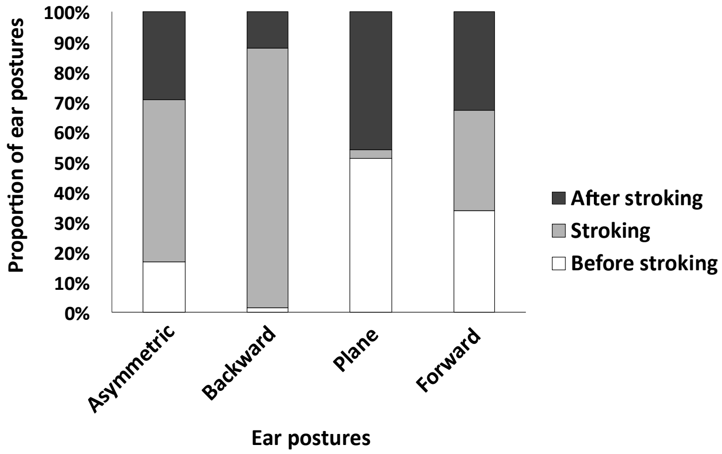

Fig. 6. Percentage of time ewes spent with each of the ear posture throughout the three stroking stages.

nificantly less frequent (P<0.05) in stroking phase compared to pre- and post-stroking. In addition, ewes spent more time with their ears backward (P<0.05) during stroking stage in comparison to pre- and post-stroking phases.

The percentage of time spent by ewes in each ear posture for the three stages is shown on Fig. 6. The ewes spent 51% of time with ears in plane position. This proportion decreased to 3% during stroking and increased to 46% during post-stroking, whereas ewes only spent 1% of their time with ears backward before stroking, 86% during stroking and 12% after stroking. Time spent with ears forward was mostly similar in all stages but ewes spent more time with asymmetrical ears (54%) during the stroking period (Fig. 6).

The frequency of ear position change per 30-second intervals is shown on Fig. 7. In comparison to pre-stroking stage, the number of ear position change was not significantly increased during stroking, however, it increased considerably (P<0.05) in post-stroking than in the stroking period (Fig. 7).

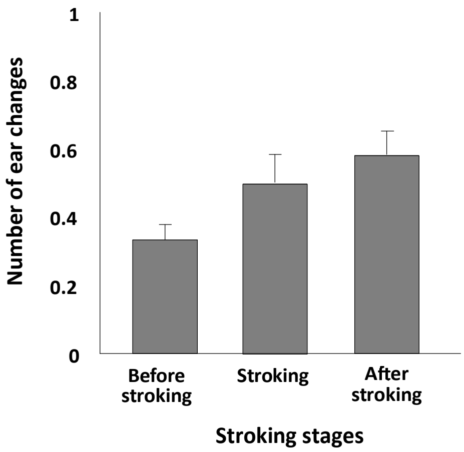

Fig. 7. Number of ear changes (mean ± SEM) by ewes in different stages of stroking.

DISCUSSION

The main purpose of this research was to evaluate the effect of stroking on ewes' positive emotional state. The stroking has significantly decreased the temperature of eyes, nose and ears of animals. In addition, it has significantly changed the facial expressions of ewes. The frequency of ear position change in ewes was significantly higher in the post-stroking stage.

Many consider that it is not possible to understand or even measure the animals' emotional states because of their subjective nature. If animal emotions are identified rapidly and effortlessly, discomfort may be avoided. Therefore, the development of emotional indicators that are both realistic and visible is crucial (Proctor & Carder, 2015a,b; Machado & Silva, 2020). Some authors used body changes measurement for detecting animal emotions (Proctor & Carder, 2014; 2015b; Lambert & Carder, 2017; 2019). Biossy *et al.* (2011) measured emotional state of sheep in relation to ear postures and found that positive states, like a feed reward made animals move their ears more forward. In addition, the study of Tamioso *et al.* (2018) indicated that stroking can elicit positive emotions in sheep. Tamioso *et al.* (2018) found more frequently horizontal (forward) ears in sheep during stroking (Tamioso *et al.*, 2018). However, in the present study, the forward and asymmetrical ear postures were not significantly affected by stroking animals' body. This may be due to the fact that studied sheep had large and pending ears as shown on Fig. 2. In a study of Proctor & Carder (2014), stroking cow's body was used to measure ear postures. The authors found that the frequency of ear posture change increased during stroking in comparison to pre-stroking phase. The results of the present study are compatible with those of Proctor and Carder (2014) in that the ear posture change was only increased in the post-stroking phase. However, in a previous research with sheep, the frequency of ear changes was found to be lower (Reefmann *et al.*, 2009).

Considering facial expressions, little information has been previously published on positive faces in animals. It was previously found that ruminants half-close or completely close their eyes during positive stimulation. Tomioso *et al.* (2018) and Proctor & Carder (2015b) found a higher percentage of closed and half-closed eyes in both sheep and cows during stroking or brushing. Therefore, the results of the present study are in agreement with data from these studies. Most of the current study findings are in line with previously reported findings regarding the ear posture changes and facial expressions (Sandem *et al.*, 2006; Reefmann *et al.*, 2009; Proctor & Carder, 2014; 2015; Tamioso *et al.*, 2018). Tail wagging in sheep was also used as an indicator of positive emotions (Tamioso *et al.*, 2018). It is quite difficult to undertake a study measuring tail wagging in Iraqi sheep as their tail is a bulk of fat and different from most European animals. According to the literature, it is clear that stroking similarly to allogrooming is pleasant for animals. In that case, animals feel more relaxed than excited (Schmied *et al.*, 2010; Proctor & Carder, 2014; 2015b; Tamioso *et al.*, 2018).

The results about peripheral temperatures of the present study showed that stroking decreased the eye, ear and nasal temperatures in sheep. So far, few studies were undertaken to measure peripheral temperatures in order to indicate positive states in animals as most of studies were focused on negative states. Measurement of peripheral temperatures was approved to be accurate, non-invasive, and not time-consuming tool (Omóbòwálé *et al.*, 2017). Proctor & Carder (2015a) used positive, low arousal emotional state by stroking cows' body to measure nasal temperature and found that it dropped from 26.3 °C to 25.8 °C during stroking and as increased again to 26.4 °C during the post-stroking stage. In another study, Hussein (2018) affirmed that stroking sheep' body can significantly reduce both eye and nasal temperatures. Therefore, the peripheral temperatures in the current study findings reduction during stroking agreed with these findings. The drop in peripheral temperatures may be due to the vasoconstriction of peripheral blood vessels during emotional state (Proctor & Carder, 2015a) contrary to findings of Proctor & Carder (2016) that the peripheral temperature did not drop during the positive stimulus such as offering concentrate instead of standard feed, although it decreased after the treatment. Previous data found that peripheral temperatures decreased during both negative and positive emotional stimuli (Stewart *et al.*, 2005; Proctor & Carder, 2015a; 2016). Therefore, much training is required for the trainer to identify a change in negative or positive states and to compare them with other physiological states e.g. estrus, because in this period a core body temperature was reported to increase for a short period of time and in turn decreased the peripheral temperatures (Piccione *et al.*, 2003).

CONCLUSIONS

According to the results obtained from the current research, it can be concluded that stroking ewes body had significantly improved the positive emotional state in healthy sheep. The facial expressions, ear movement and peripheral temperatures can be used to detect positive emotions in small ruminants such as sheep. More research is required to differentiate the effect of stroking different body parts on positive emotions of sheep. According to the findings of the present research, farmers and sheep owners can easily detect whether their sheep welfare is good or poor.

ACKNOWLEDGEMENTS

The authors are thankful to the ewes' owners who helped them stroking ewes' body throughout the experiment. Gratitude to the Environmental Sciences Department for helping the authors to undertake the research.

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Paper received 21.12.2022; accepted for publication 20.03.2023

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