Acute stress assessment using infrared thermography in fattening rabbits reacting to handling under winter and summer conditions

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Abstract

Aim of study: This study assesses acute stress by measuring, through infrared thermography in summer and winter, the temperature of the eye, outer ear, inner ear and nose in 40 fattening rabbits before and after handling.

Area of study: Seville (Spain).

Material and methods: Body thermographic temperatures were recorded during a 38-day fattening period twice weekly and twice a day, before and after the handler held the rabbits in their arms for one minute. Ambient temperature and relative humidity were also recorded, and their influence on body temperatures was assessed. For each anatomical part, the variation of the temperature between the handled and undisturbed rabbit, and the differential temperature between the anatomical part in the undisturbed rabbit and the ambient temperature were calculated.

Main results: The variation in temperatures between handled and undisturbed rabbits ranged from 0.25±0.041 °C for eye to 3.09±0.221 °C for outer ear in summer and -0.41±0.182 °C for nose to 2.09±0.178 °C for outer ear in winter. The day of the fattening period influenced all the temperature traits during summer and winter, except for the inner ear in winter. In summer, unlike winter, the temperature variation at the end of fattening period between handled and undisturbed rabbits was lower than at weaning (-0.04 to 1.94 °C vs. 0.54 to 5.52 °C, respectively). The temperatures in undisturbed rabbits were correlated with ambient temperature.

Research highlights: Measuring body temperature with infrared thermography is a useful tool to evaluate acute stress in handled rabbits, with the inner ear and eye the most reliable body parts for measuring it.

Additional key words: temperature; welfare; thermoregulation.

Abbreviations used: DIF (differential); H (handled); RH (relative humidity); TEMP (room temperature); U (undisturbed); VAR (variation).

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Introduction

Worldwide, rabbits make up the fourth most common form of animal production, and they are the second commonest species in the European Union, with nearly 160 million animals slaughtered per year for meat production in 2017. More than three quarters of all rabbit farms are found in Mediterranean countries such as Italy, Spain and France (FAOSTAT, 2019).

Current systems of intensive rabbit breeding, as in other species, are highly dependent on humans, since they must feed them and control their photoperiod, reproduction, etc. Moreover, one of the biggest breeding handicaps is that rabbits continue to perceive humans as predators, and are predisposed to associate their presence with negative stimuli, which constitutes a factor of stress and fear (Trocino & Xiccato, 2006), since they are prey animals by nature (Benato et al., 2019). Shyness is one of the main attributes of rabbits; they are elusive and independent (Trocino & Xiccato, 2006), which makes it harder for us to perceive their fear or acute stress.

Stress is an adaptive phenomenon in an animal’s response to the changes that occur in its environment (Veissier & Boissy, 2007) and it involves the organism’s
response to a stimulus which triggers activation of the hypothalamic–pituitary–adrenocortical axis (HPA) and sympato-adrenomedullary system (Möstl & Palme, 2002). Different studies have shown that this occurs by activating a series of behavioral changes, mainly physiological and escape behavior (Temple et al., 2014). On farms, rabbits are subjected daily to pressure when handled, which causes them stress (Xu, 1996), and this stress could lead to lower immune competence and higher susceptibility to disease (Glaser & Kiecolt-Glaser, 2005).

A reduction in rabbits’ fear of humans can be achieved by accustoming rabbits to human contact. This fundamentally happens when socialization takes place, at around 10-20 days of life, although it can even be carried out from birth (Zucca et al., 2012). The habituation process caused by a routine repeated over time stimulates the HPA less, and subsequent reactions are milder (Grissom & Bhatnagar, 2009). Stress mechanisms can be compared to the physiological mechanism of exercise; the more training, the better the results and the lower the stress (Temple et al., 2014).

Among the physiological changes caused by fear, where the hypothalamus plays a key integrating role in the endocrine and nervous system (Minton, 1994), there is the release of several hormones. The most important of these are catecholamines (especially adrenaline and noradrenaline), corticotropin-releasing hormone, adrenocorticotropic hormone and corticosteroids (in rabbits, mainly corticosterone and cortisol) released as a consequence of the alteration of internal homeostasis, which produce, among other effects, an increase in body temperature (Kataoka et al., 2014) as a consequence of peripheral and abdominal vasoconstriction. These physiological alterations are rapid responses which guarantee the survival of the animals (Wingfield et al., 1997).

In countries highly specialized in rabbit meat production, such as those in Mediterranean countries in Western Europe, rabbits are raised in cages under controlled environment housing systems (Lebas et al., 1997). Here, the correct regulation of temperature and humidity are key aspects to avoid the animals suffering thermal stress. The optimum temperature in rabbit farms is between 15-19 and 21-22 °C for the fattening phase (Ferré & Rosell, 2000; Coureauad et al., 2015), and 16-20 °C for the does, with a relative humidity of 55-60% (Ferré & Rosell, 2000).

Infrared thermography is the recording of the radiation emitted by a body surface using an infrared camera. It is a non-invasive, quick technique used to assess physiological states and in cases of pathologies linked to temperature changes, and its use has been confirmed in many domestic and wild species (Travain et al., 2015; Sánchez et al., 2016). It is used to evaluate and monitor the temperature of rabbits in nests with different materials (Silva et al., 2014) and to estimate live weight in fattening rabbits (Silva et al., 2015), among other applications. Although works have been published on human influence in the handling of kits (Bilkó & Altbäcker, 2000; Csatódi et al., 2005) and mature rabbits (Podberscek et al., 1991), no studies on stress have described the handling of fattening rabbits without previous human manipulation during lactation.

The objective of this work was therefore to evaluate the levels of stress in fattening rabbits, measured by thermography temperature in four body parts (eye, inner ear, outer ear and nose) in two situations, before and after being handled by humans in two meteorological seasons (winter and summer).

Material and methods

Animals and husbandry

We used common Spanish agouti-coated domestic meat-oriented rabbits belonging to a strain kept at the Teaching Farm at the Higher Technical School of Agricultural Engineering of the University of Seville (Spain). The genetic characterization (Emam et al., 2016a,b) and productive performance (González-Redondo, 2016) of this nucleus have been previously described. Overall, the rabbits were phenotypically similar to the recently recognized autochthonous rustic breed “Antiguo Pardo Español” (Cañón, 2015).

The rabbits were individually housed in polyvalent wire-mesh cages measuring 90 × 40 × 30 cm (length, width and height), located in a conventional closed facility with natural ventilation (geographic coordinates: 37° 21’ 36.3” N and 5° 56’ 23.9” W; 11 m a.s.l.). The animals were subjected to a natural photoperiod.

The rabbits were fed a commercially-produced balanced diet (15.0% crude protein and 15.5% crude fiber) and water ad libitum.

The rabbits were not subjected to any socialization before the experiment, and were only exposed once a day to the person who filled the feeders and supervised the experimental stock. This was the same person that performed the trial in the experimental period.

Collecting the temperature data

A total of 40 weaning rabbits, with an age of 28 days old, were analyzed during a 38-day fattening period in two different seasons: summer (April 14th to May 22nd, 2015; n = 23 rabbits) and winter (January 19th to February 23rd, 2016; n = 17 rabbits). An average of 24 records per rabbit...
was taken, amounting to a total of 456 records. The stress levels of the animals were assessed with eye, outer ear, inner ear and nose temperature measurements. Temperature samples were collected twice a week (each day will be called “record collection day”) and twice each day: the first was taken at 11:00 h when the animal was undisturbed (U) in its own cage from the previous day and the second was taken with the rabbit was held in arms (H), ten minutes after the first temperature was taken. The rabbits were held in arms for about one minute. Temperature images of the undisturbed rabbits were taken with the cages open, without touching the animals and at a distance of 100 cm from their bodies. The whole procedure for the entire experimental stock took about 2½ hours each day and was always carried out by the same person. The temperature images were taken with a FLIR i7 camera, following the instructions given by Bartolomé et al. (2013). To calibrate the camera results, room temperature and relative humidity were recorded with a digital thermos-hygrometer (Extech® 44550) every time an infrared body temperature sample was taken, so each infrared temperature had a corresponding humidity and room temperature.

The experiment was carried out in accordance with the Spanish legislation (RD 53/2013; BOE, 2013) and the Directive 2010/63/EU on the protection of animals used for scientific purposes (OJ, 2010).

Room and infrared temperatures

In order to evaluate the environmental conditions and it relationship with the infrared temperature the following data were recorded:

— TEMP, U: Room temperature (ºC) taken at the precise moment when the infrared temperatures were taken in undisturbed rabbits.
— TEMP, H: Room temperature (ºC) taken at the precise moment when the infrared temperatures were taken in handled rabbits.
— RH, U: Relative humidity (%) taken at the precise moment when the infrared temperatures were taken in undisturbed rabbits.
— RH, H: Relative humidity (%) taken at the precise moment when the infrared temperatures were taken in handled rabbits.
— Eye, U: Infrared eye temperature (ºC) with the rabbit undisturbed.
— Eye, H: Infrared eye temperature (ºC) with the rabbit handled.
— Inner ear, U: Infrared inner ear temperature (ºC) with the rabbit undisturbed.
— Inner ear, H: Infrared inner ear temperature (ºC) with the rabbit handled.
— Outer ear, U: Infrared outer ear temperature (ºC) with the rabbit undisturbed.
— Outer ear, H: Infrared outer ear temperature (ºC) with the rabbit handled.
— Nose, U: Infrared nose temperature (ºC) with the rabbit undisturbed.
— Nose, H: Infrared nose temperature (ºC) with the rabbit handled.
— Eye VAR = Eye, H - Eye, U.
— Inner ear VAR = Inner ear, H - Inner ear, U.
— Outer ear VAR = Outer ear, H - Outer ear, U.
— Nose VAR = Nose, H - Nose, U.
— DIF Eye-TEMP = Eye, U – TEMP, U.
— DIF Inner ear-TEMP = Inner ear, U - TEMP, U.
— DIF Outer ear-TEMP = Outer ear, U - TEMP, U.
— DIF Nose-TEMP = Nose, U - TEMP, U.

Statistical analyses

The descriptive statistics for each trait are shown in Tables 1 and 2. The evolution of the differential temperatures (ºC) for eye, inner ear, outer ear and nose during summer and winter are represented in Fig. 1. A General Linear Model was used to study the potential risk factors (sex, day and each rabbit for each season; winter and summer) that could most influence body temperature during the experimental periods in U and H rabbits (Table 3). This was followed by a Duncan post-hoc test to study the differences between the first and the last days studied and between the same day in summer and winter (Table 4). Finally, to study the correlation between all the traits studied in summer and in winter in undisturbed and handled rabbits, Pearson correlations were carried out (Table 5). All the procedures were analyzed using the Statistica 8.0 package for Windows.

Results

Temperature data

The rabbits showed a mean ranging from 29.10±0.384 ºC for outer ear temperature to 37.83±0.048 ºC for eye temperature and from 32.19±0.305 ºC for outer ear temperature to 38.08±0.039 ºC for eye temperature in undisturbed and handled rabbits, respectively, in summer (Table 2). In winter, the mean temperature ranged from 16.22±0.129 ºC for outer ear temperature to 36.85±0.060 ºC for eye temperature and from 18.30±0.219 ºC for outer ear temperature to 37.27±0.045 ºC for eye temperature in undisturbed and handled rabbits, respectively. For the variation in temperatures between handled and undisturbed
Table 1. Number (n), mean, minimum (Min), maximum (Max) and standard deviation (s.d.) of environmental temperature (ºC) and relative humidity (%) during the experimental periods (summer 2015 and winter 2016) recorded when the rabbits were handled (H) and undisturbed (U).

|                  | Summer (n = 269) | Winter (n = 187) |
|------------------|------------------|------------------|
|                  | Mean±s.e. | Min | Max | s.d. | Mean±s.e. | Min | Max | s.d. |
| Temperature, U (ºC) | 24.14±0.332 | 16.50 | 31.78 | 5.44 | 14.09±0.107 | 10.70 | 17.90 | 1.47 |
| Temperature, H (ºC) | 25.02±0.212 | 20.20 | 32.10 | 3.47 | 14.40±0.093 | 11.30 | 16.70 | 1.27 |
| Relative humidity, U (%) | 54.20±0.863 | 25.00 | 80.00 | 14.15 | 69.31±0.781 | 42.00 | 84.00 | 10.68 |
| Relative humidity, H (%) | 49.17±0.766 | 27.60 | 73.00 | 12.57 | 69.50±0.788 | 42.00 | 84.00 | 10.77 |

Table 2. Number (n), mean, minimum (Min), maximum (Max) and standard deviation (s.d.) of the body temperatures (ºC) registered during the experimental periods (summer 2015 and winter 2016) in undisturbed (U) and handled (H) rabbits.

|                  | Summer (n = 269) | Winter (n = 187) |
|------------------|------------------|------------------|
|                  | Mean±s.e. | Min | Max | s.d. | Mean±s.e. | Min | Max | s.d. |
| Eye, U | 37.83±0.048 | 33.33 | 40.03 | 0.79 | 36.85±0.060 | 33.40 | 38.37 | 0.82 |
| Eye, H | 38.08±0.039 | 34.60 | 39.57 | 0.64 | 37.27±0.045 | 35.70 | 39.75 | 0.61 |
| Inner ear, U | 30.58±0.350 | 20.95 | 40.55 | 5.74 | 17.50±0.148 | 13.73 | 27.95 | 2.03 |
| Inner ear, H | 32.85±0.320 | 22.00 | 39.35 | 5.25 | 18.78±0.226 | 14.00 | 34.50 | 3.09 |
| Outer ear, U | 29.10±0.384 | 19.75 | 40.50 | 6.30 | 16.22±0.129 | 12.90 | 25.75 | 1.76 |
| Outer ear, H | 32.19±0.305 | 22.25 | 39.00 | 5.00 | 18.30±0.219 | 13.95 | 32.40 | 3.00 |
| Nose, U | 33.71±0.199 | 3.10 | 39.15 | 3.27 | 31.74±0.162 | 22.45 | 36.50 | 2.21 |
| Nose, H | 34.72±0.113 | 29.20 | 38.45 | 1.85 | 31.33±0.154 | 23.10 | 36.75 | 2.11 |
| Eye VAR | 0.25±0.041 | -2.00 | 4.57 | 0.67 | 0.42±0.053 | -1.23 | 2.65 | 0.73 |
| Inner ear VAR | 2.27±0.227 | -9.05 | 16.30 | 3.73 | 1.28±0.194 | -10.40 | 17.60 | 2.65 |
| Outer ear VAR | 3.09±0.221 | -8.40 | 15.55 | 3.63 | 2.09±0.178 | -6.85 | 14.75 | 2.44 |
| Nose VAR | 1.01±0.159 | -4.10 | 29.65 | 2.60 | -0.41±0.182 | -9.20 | 7.25 | 2.49 |
| DIF Eye-TEMP | 13.69±0.313 | 4.05 | 20.86 | 5.14 | 22.76±0.110 | 17.45 | 26.87 | 1.51 |
| DIF Inner ear-TEMP | 6.43±0.336 | -4.50 | 19.70 | 5.51 | 3.42±0.124 | -0.50 | 12.95 | 1.69 |
| DIF Outer ear-TEMP | 4.96±0.355 | -5.95 | 17.05 | 5.82 | 2.13±0.102 | -1.20 | 10.75 | 1.40 |
| DIF Nose-TEMP | 9.89±0.114 | -0.40 | 19.10 | 187 | 17.66±0.193 | 7.55 | 24.25 | 2.64 |

VAR: temperature in handled rabbits – temperature in undisturbed rabbits. TEMP: room temperature for undisturbed rabbits. DIF Eye-TEMP = Eye, U – TEMP, U. DIF Inner ear-TEMP = Inner ear, U – TEMP, U. DIF Outer ear-TEMP = Outer ear, U – TEMP, U. DIF Nose-TEMP = Nose, U – TEMP, U.
rabbits, the mean values ranged from 0.25±0.041 ºC for eye temperature to 3.09±0.221 ºC for outer ear temperature in summer and -0.41±0.182 ºC for nose temperature to 2.09±0.178 ºC for outer ear temperature in winter. Finally, in the case of the differential temperatures between the undisturbed rabbits and the room temperature, the mean values ranged from 13.69±0.313 ºC for eye temperature to 4.96±0.355 ºC for ear outer temperature in summer and from 22.76±0.110 ºC for eye temperature to 2.13±0.102 ºC for ear outer temperature in winter.

Environmental effects

The environmental effects that could most influence temperature were studied (Table 3). The day of the fattening period produced statistically significant differences for all the temperature traits collected during the summer and winter (except inner ear in winter). The individual rabbit was only significant for nine and three traits in summer and winter, respectively. Sex was not statistically significant in all the traits in both seasons, except for the variation in temperature between handled and undisturbed rabbits for the eye in winter.

Evolution of the temperatures

During the summer, for all the temperature traits, there were significant differences between the first day experiment at weaning (undisturbed and handled animals) and the last day of the fattening period (Table 4). At the end of the fattening period, all the values for variations in temperature were lower than at weaning. In winter, this only happened in the nose temperature variations, but there was no significant difference between the first and last day of the fattening period. The rabbit’s temperature in all the traits studied during the summer was always higher than in winter. All the temperature variations between handled and undisturbed rabbits had a negative trend during summer (Fig. 1), in spite of the rise in room temperature. This did not happen in the same way in winter with the exception of the inner ear temperature trait.

Correlations between temperatures

The eight traits studied and their differentials correlated to a medium-high degree (Table 5). In summer, the highest correlation was found between inner ear and outer
Table 3. General Lineal Model analysis of the environmental effects that could influence body temperature (ºC) most during the experimental periods (summer 2015 and winter 2016) in undisturbed (U) and handled (H) rabbits.

|                | Summer       | Winter       |
|----------------|--------------|--------------|
|                | Sex          | Day of the fattening period | Rabbit | Sex          | Day of the fattening period | Rabbit |
| Eye, U         | n.s.         | ***           | n.s.     | ***           | n.s.         |   |
| Eye, H         | n.s.         | ***           |   | n.s.         | ***           | n.s. |
| Inner ear, U   | n.s.         | ***           | n.s.     | ***           | n.s.         |   |
| Inner ear, H   | n.s.         | ***           | n.s.     | ***           | n.s.         |   |
| Outer ear, U   | n.s.         | ***           | n.s.     | ***           | n.s.         |   |
| Outer ear, H   | n.s.         | ***           | n.s.     | ***           | n.s.         |   |
| Nose, U        | n.s.         | ***           | n.s.     | ***           | n.s.         |   |
| Nose, H        | n.s.         | ***           | n.s.     |   | n.s.         | ***           |   |
| Eye VAR        | n.s.         | ***           | n.s.     | ***           | n.s.         |   |
| Inner ear VAR  | n.s.         | ***           | n.s.     |   | n.s.         | ***           |   |
| Outer ear VAR  | n.s.         | ***           | n.s.     |   | n.s.         | ***           |   |
| Nose VAR       | n.s.         | ***           | n.s.     |   | n.s.         | ***           |   |
| DIF Eye-TEMP   | n.s.         | ***           | n.s.     |   | n.s.         | ***           |   |
| DIF Inner ear-TEMP | n.s.   | ***           | n.s.     |   | n.s.         | ***           |   |
| DIF Outer ear-TEMP | n.s.   | ***           | n.s.     |   | n.s.         | ***           |   |
| DIF Nose-TEMP  | n.s.         | ***           | n.s.     |   | n.s.         | ***           |   |

*: p<0.05, **: p<0.01, ***: p<0.001, n.s.: not significant. VAR: temperature in handled rabbits – temperature in undisturbed rabbits. TEMP: room temperature for undisturbed rabbits. DIF Eye-TEMP = Eye, U – TEMP, U. DIF Inner ear-TEMP = Inner ear, U – TEMP, U. DIF Outer ear-TEMP = Outer ear, U – TEMP, U. DIF Nose-TEMP = Nose, U – TEMP, U.

Table 4. Duncan post-hoc least squared means analysis of the environmental risk factor in temperature traits studied (ºC, mean) during the experimental periods (summer 2015 and winter 2016) in undisturbed and handled rabbits.

|                | Weaning                  | End of the fattening period |
|----------------|--------------------------|----------------------------|
|                | Undisturbed | Handled | Variation | Undisturbed | Handled | Variation |
|                | Summer (n = 269)        |           |           | Winter (n = 187)         |           |           |
| Eye            | 37.25 aB 37.80 aB 0.54 b | 38.11 bB 38.16 bB 0.52 a |
| Inner ear      | 26.30 aB 29.81 aB 3.51 bB | 35.28 bB 36.33 bB 1.06 a |
| Outer ear      | 24.48 aB 30.01 aB 5.52 bB | 33.82 bB 35.76 bB 1.94 a |
| Nose           | 31.66 aB 34.73 aB 3.07 bB | 35.38 bB 35.75 bB -0.04 a |

Different superscript upper-case letters show significant differences (p<0.05) in the same experiment day between summer and winter. Different superscript lower-case letters show significant differences (p<0.05) within the same season between the weaning and the end of the fattening period. Variation: temperature in handled rabbits - temperature in undisturbed rabbits.
ear temperature ($r = 0.97$) and the lowest between eye temperature in undisturbed rabbits and outer ear and nose temperature taken in handled animals ($r = 0.53$); 27.51% of the Pearson correlations in summer were higher than 0.59, 29.11% were between 0.59 and 0.40 and 52.38% were lower than 0.40. In winter, the Pearson correlations were lower, ranging from 0.07 (eye temperature in undisturbed and handled rabbits). 13.68% of the Pearson correlations in winter were higher than 0.59, 12.11% were between 0.59 and 0.40 and 74.21% were lower than 0.40.

**Discussion**

When the animal is faced with an alarm, acute stress is activated, which is characterized as a short response over time (Dickens & Romero, 2013). Body temperature also increases, which is particularly problematic in species with deficient heat tolerance, such as rabbits. This increase in temperature occurs when the sympathetic autonomic nervous system is stimulated, releasing the neurotransmitters adrenaline and noradrenaline (catecholamines) in the adrenergic synapses, in order to obtain energy to face the emergency (Duval et al., 2010). In the case of the rabbit, between 1 and 2 seconds after a stressful effect begins (Manteca, 1998) a primitive escape reaction occurs, releasing catecholamines (Kuchel, 1991) into the bloodstream, which makes the body temperature rise for a short time, since its function is to favor hepatic glycogenesis (Greco & Stabenfeldt, 1997). It is a short-term response, since catecholamines have a half-life of just a few minutes in the blood (Peaston & Weinkove, 2004). This circumstance allows the animals to deal with the fear situation effectively (Lattin & Romero, 2014). Infrared thermography can detect this acute response to stress, replacing traditional systems of measuring plasma catecholamines as responses of the autonomic nervous system to assess the animals’ welfare (Stewart et al., 2005).

The increase in temperature through stress has been evaluated by using infrared thermography in different anatomical parts in various studies, in the eye in horses (Valera et al., 2012), in cows (Stewart et al., 2007), and in dogs (Travain et al., 2015), as well as in the skin of pigs (Warriss et al., 2006). Olivas & Villagrá (2013) showed that rectal temperature can also be used to assess fear or acute stress in rabbits. The results of the present study show an increase in infrared temperature (measured in eye, inner ear and outer ear both in winter and summer) between the initial state without previous handling and after handling the animal and holding it. These anatomical parts are therefore key points of interest for measuring stress in this species, since stress can lead to hyperthermia. Higher temperatures induced by stress occurred in the eye, inner ear and outer ear. In the same context, De Lima et al. (2013), in a study on heat stress in rabbits, found that the highest temperatures were detected by infrared thermography in the eye, followed by inner ear, outer ear and nose.

The greatest temperature variations between the initial conditions without handling and after handling occurred in the measurements of the inner ear, so this point of interest should be the reference for assessing stress in rabbits by temperature measured with thermography, as stated in Ludwig et al. (2007). In the case of the nose, different infrared temperature data were obtained in winter and summer. This could be due to the humidity in the nose, which can differ according to the room temperature in the different seasons. This humidity can therefore alter the real value of the infrared temperature measured (Luzi et al., 2007).

Contrary to our findings in this work, the studies carried out by Ludwig et al. (2007) on measuring stress by thermography in rabbits show that there is a decrease in the temperature of the eye and outer ear following a stressful event, and that corticosterone also increases in the bloodstream. This occurs by activation of the hypothalamic-pituitary-adrenal axis, which occurs 2-10 minutes post stress (Nelson, 2000). However, this does not happen, or only happens slightly, in the first phase of stress (alarm), in which the stimulation of the autonomic nervous system occurs (Nelson, 2000). When there is a short, albeit stressful handling, the rabbits’ reaction is very intense, producing an increase in corticosterone from 60 seconds after the start of handling (Gascon & Verde, 1987).

The degree of hyperthermia induced by stress recorded by us in the eye was not significantly affected by room temperature, as verified in Long et al. (1990). This also happened with the variation in humidity, although in this case, the difference was greater in winter. However, in the inner and outer ear, the room temperature was correlated with the increase in the infrared temperature variation between measurements taken before and after handling. The difference in the temperature values for each anatomical point studied is due to their different vascularization and to the influence of the ambient temperature. The outer ears, like the nose, are used by rabbits to dissipate heat (Fayez et al., 1994). To regulate heat better, rabbits have larger ears in warmer geographic areas (Ferreira et al., 2015). Rabbıts with larger ears have lower respiratory rates with high ambient temperatures (Zeferino et al., 2011). In the present trial, the maximum temperature induced by stress occurred in the eye, with 39.57 °C in summer and 39.75 °C in the outer ear in winter; as a result, hyperthermia by induction of stress was not pathological for the
rabbits tested, as shown in Ardiaca et al. (2010). Above that temperature, cell membranes begin to be destroyed by denaturing proteins (Bowler & Manning, 1994).

The state of fear or stress in the animals in this trial could be due to the fact that they were not manipulated early, as indicated in Price (2002), but rather directly after weaning. In fact, handling during lactation reduces rabbits’ fear of humans (Bilkó & Altbäcker, 2000). Csatádi et al. (2005) also show that early handling in rabbit offspring significantly reduces the stress caused by human presence. Olivas & Villagrá (2013) reported that manipulation per se does not cause hyperthermia, but that it is caused by the stressors of the study. Cabezas et al. (2007) confirmed that in captivity, wild rabbits show fear episodes and flight reactions due to human presence and handling.

The temperature of the undisturbed rabbit’s anatomical parts tested was correlated with the room temperature, as found by Cervera & Fernández-Carmona (1998), but not with the relative humidity. The room temperature where the animals are kept is a key condition for the regulation of a rabbit’s internal temperature (Sanmiguel & Díaz, 2011). In summer, there was a decrease in the temperature difference of the monitored anatomical parts during the fattening period, between the first and last days of the experiment; it could therefore be said that there was a process of gradual adjustment to human handling, which did not happen in winter. The greater differences between the temperature of the handled and unhandled rabbits occurring in summer at the beginning of the fattening period versus the smaller differences at the end could be due to the thermal stress suffered by the rabbits in warm environments, as their age and body weight increase throughout the fattening time. In other words, the animals in the first stages of fattening do not suffer heat stress and, consequently, the differences between the temperature when they are handled and the initial unhandled temperature are

Table 5. Pearson correlation among all the traits studied (temperature in °C and relative humidity in %) in summer 2015 (above the diagonal) and in winter 2016 (below the diagonal) in undisturbed (U) and handled (H) rabbits.

|                | Eye, U  | Eye, H  | Inner ear, U | Inner ear, H | Outer ear, U | Outer ear, H | Nose, U  | Nose, H  | Eye VAR | Inner ear VAR |
|----------------|---------|---------|--------------|--------------|--------------|--------------|----------|----------|---------|--------------|
| Eye, U         | 0.58*   | 0.61*   | 0.49*        | 0.62*        | 0.53*        | 0.58*        | -0.63*   | -0.26*   |         |              |
| Eye, H         | 0.95*   | 0.62*   | 0.68*        | 0.63*        | 0.68*        | 0.71*        | 0.27*    | 0.00     |         |              |
| Inner ear, U   | 0.36*   | 0.58*   | 0.77*        | 0.97*        | 0.80*        | 0.64*        | 0.69*    | -0.13*   | -0.45*  |              |
| Inner ear, H   | 0.07    | 0.11    | 0.53*        | 0.79*        | 0.95*        | 0.59*        | 0.66*    | 0.08     | 0.22*   |              |
| Outer ear, U   | 0.35*   | 0.58*   | 0.88*        | 0.52*        | 0.82*        | 0.66*        | 0.71*    | -0.13*   | -0.38*  |              |
| Outer ear, H   | 0.52*   | 0.24*   | 0.24*        | 0.23*        | 0.29*        | 0.60*        | 0.68*    | 0.02     | 0.10    |              |
| Nose, U        | 0.37*   | 0.15*   | 0.26*        | 0.13         | 0.30*        | 0.23*        | 0.61*    | -0.13*   | -0.15*  |              |
| Nose, H        | 0.35*   | 0.17*   | 0.31*        | 0.15         | 0.36*        | 0.44*        | 0.34*    | 0.06     | -0.14*  |              |
| Eye VAR        | -0.69*  | 0.07    | -0.20*       | 0.12         | -0.15*       | 0.26*        | -0.22*   | -0.03    | 0.31*   |              |
| Inner ear VAR  | -0.20*  | 0.67*   | -0.15*       | 0.76*        | -0.07        | 0.08         | -0.04    | -0.06    | 0.29*   |              |
| Outer ear VAR  | -0.11   | 0.81*   | 0.07         | 0.80*        | -0.01        | 0.09         | -0.04    | -0.05    | 0.20*   | 0.87*        |
| Nose VAR       | -0.04   | 0.01    | 0.03         | 0.01         | 0.04         | 0.16*        | -0.60*   | 0.55*    | 0.18*   | -0.01        |
| DIF Eye-TEMP   | 0.24*   | 0.34*   | 0.70*        | 0.31*        | 0.50*        | 0.08         | 0.30*    | 0.06     | -0.20*  | -0.17*        |
| DIF Inner ear-TEMP | 0.20* | 0.31*   | 0.51*        | 0.26*        | 0.59*        | 0.11         | 0.37*    | 0.08     | -0.13  | -0.08        |
| DIF Outer ear-TEMP | 0.19* | -0.10   | -0.10        | -0.09        | -0.10        | 0.06         | 0.83*    | 0.08     | -0.16*  | -0.03        |
| DIF Nose-TEMP  | 0.32*   | -0.33*  | -0.36*       | -0.32*       | -0.43*       | 0.05         | 0.19*    | -0.16*   | -0.32*  | -0.10        |
| TEMP, U        | 0.23*   | 0.40*   | 0.57*        | 0.37*        | 0.64*        | 0.24*        | 0.01     | 0.36*    | -0.05*  | -0.01        |
| RH, U          | 0.05    | 0.34*   | 0.50*        | 0.28*        | 0.61*        | 0.12         | 0.18*    | 0.33*    | 0.04    | -0.06        |
| TEMP, H        | 0.30*   | 0.44*   | 0.63*        | 0.40*        | 0.72*        | 0.31*        | 0.10     | 0.38*    | -0.08*  | -0.02        |
| RH, H          | 0.09    | 0.35*   | 0.52*        | 0.30*        | 0.63*        | 0.15*        | 0.15*    | 0.35*    | 0.03    | -0.05        |

VAR: temperature in handled rabbits – temperature in undisturbed rabbits. TEMP: room temperature. RH: relative humidity. DIF Eye-TEMP = Eye, U − TEMP, U. DIF Inner ear-TEMP = Inner ear, U − TEMP, U. DIF Outer ear-TEMP = Outer ear, U − TEMP, U. DIF Nose-TEMP = Nose, U − TEMP, U. *: p<0.05
relatively large. However, in the final stage of fattening, heat stress is produced in the rabbits and, consequently, the initial temperature is higher, with a naturally smaller difference between the temperature of the handled animal and the initial temperature. This fact has been observed in rabbits (Daader et al., 2018) and in other species (Soleimani et al., 2008; Collier et al., 2019): as the animals are older and gain weight, they become more sensitive to thermal stress, and their body temperature rises. As a consequence, for rabbit production, this implies that on farms, it is heat stress that needs to be controlled more strictly as the fattening period progresses, and that handling does not increase thermal stress in rabbits.

The room temperature in which the animals are kept is the key factor in the rabbits’ ability to regulate their temperature (Cervera & Fernández-Carmona, 1998), and they thermoregulate more efficiently in lower temperatures (Lebas et al., 1997). Starting at 24 °C, weaned rabbits during the fattening period begin to have breathing problems, with fatigue, increased heart rate, lack of appetite and decreased basal metabolism (Samoggia, 1987). Although our room temperatures were slightly higher, none of these effects were observed in the current trial, perhaps due to this breed’s adaptation to the warm Spanish climate (Cañón, 2015) and due to the rabbits’ phenotypic plasticity (Dalmau et al., 2015).

Energy is required for the flight reaction, which justifies the increase in temperature (Duval et al., 2010), and this need is greater in winter than in summer (Samoggia, 1987). The animals’ ability to generate energy for flight after handling, demonstrated through variations in body temperature, was much greater in winter than in summer. Before the response to stress, the lower metabolic activity in summer (Okab et al., 2008) and the greater need for

| Table 5. Continued |
|-------------------|
| **Outer ear VAR** | **Nose VAR** | **DIF Eye-TMP** | **DIF Inner ear-TMP** | **DIF Outer ear-TMP** | **DIF Nose-TMP** | **TEMP, U** | **RH, U** | **TEMP, H** | **RH, H** |
| Eye, U            | -0.34*       | -0.35*        | 0.20*               | 0.25*                | -0.10           | -0.32*      | 0.45*      | -0.54*      | 0.67*      | -0.51*      |
| Eye, H            | -0.17*       | -0.23*        | 0.27*               | 0.33*                | -0.03           | -0.32*      | 0.38*      | -0.55*      | 0.69*      | -0.56*      |
| Inner ear, U      | -0.58*       | -0.31*        | 0.53*               | 0.57*                | -0.14*          | -0.45*      | 0.52*      | -0.76*      | 0.81*      | -0.70*      |
| Inner ear, H      | -0.07        | -0.27*        | 0.48*               | 0.55*                | 0.03            | -0.27*      | 0.33*      | -0.67*      | 0.77*      | -0.69*      |
| Outer ear, U      | -0.61*       | -0.32*        | 0.50*               | 0.60*                | -0.13*          | -0.45*      | 0.52*      | -0.78*      | 0.83*      | -0.71*      |
| Outer ear, H      | -0.04        | -0.27*        | 0.48*               | 0.55*                | 0.01            | -0.30*      | 0.36*      | -0.73*      | 0.82*      | -0.74*      |
| Nose, U           | -0.31*       | -0.82*        | 0.31*               | 0.38*                | 0.25*           | -0.29*      | 0.36*      | -0.55*      | 0.62*      | -0.50*      |
| Nose, H           | -0.30*       | -0.05         | 0.34*               | 0.41*                | -0.03           | -0.33*      | 0.39*      | -0.70*      | 0.75*      | -0.69*      |
| Eye VAR           | 0.25*        | 0.20*         | 0.02                | 0.02                 | 0.09            | 0.08        | -0.16*     | 0.11        | -0.13*     | 0.07        |
| Inner ear VAR     | 0.80*        | 0.10          | -0.14*              | -0.10                | 0.25*           | 0.31*       | -0.33*     | 0.23*       | -0.16*     | 0.10        |
| Outer ear VAR     | 0.18*        | -0.20*        | -0.28*              | 0.23*                | 0.38*           | -0.41*      | 0.34*      | -0.31*      | 0.21*      |             |
| Nose VAR          | -0.01        | -0.15*        | -0.18*              | -0.34*               | 0.13*           | -0.17*      | 0.19*      | -0.24*      | 0.13*      |             |
| DIF Eye-TMP       | 0.05         | -0.22*        | 0.96*               | 0.66*                | 0.51*           | -0.45*      | -0.37*     | 0.37*       | -0.41*     |             |
| DIF Inner ear-TMP | -0.05        | -0.26*        | 0.83*               | 0.62*                | 0.44*           | -0.38*      | -0.44*     | 0.45*       | -0.47*     |             |
| DIF Outer ear-TMP | -0.05        | -0.67*        | 0.36*               | 0.45*                | 0.85*           | -0.81*      | 0.10       | -0.11       | 0.02       |             |
| DIF Nose-TMP      | -0.09        | -0.31*        | 0.31                | 0.35*                | 0.64*           | -0.99*      | 0.37*      | -0.40*      | 0.26*      |             |
| TEMP, U           | 0.03         | 0.30*         | -0.19*              | -0.25*               | -0.55*          | -0.85*      | -0.43*     | 0.47*       | -0.32*     |             |
| RH, U             | -0.03        | 0.12          | 0.14                | 0.21*                | -0.15*          | -0.49*      | 0.53*      | -0.89*      | 0.95*      |             |
| TEMP, H           | 0.02         | 0.24*         | -0.08               | -0.11                | -0.45*          | -0.77*      | 0.96*      | 0.62*       | -0.92*     |             |
| RH, H             | -0.02        | 0.16*         | 0.08                | 0.13                 | -0.23*          | -0.57*      | 0.64*      | 0.99*       | 0.70*      |             |

VAR: temperature in handled rabbits – temperature in undisturbed rabbits. TEMP: room temperature. RH: relative humidity. DIF Eye-TMP = Eye, U – TEMP, U. DIF Inner ear-TMP = Inner ear, U – TEMP, U. DIF Outer ear-TMP = Outer ear, U – TEMP, U. DIF Nose-TMP = Nose, U – TEMP, U. *: p<0.05
energy for winter flight would account for this difference in body temperature we observed between the first day (weaning) and the last day (end of the fattening period) in each of the seasons. The difference in temperature due to stress induction is due to the fact that individuals in the populations differ naturally in their physiological responses and, therefore, each animal’s capacity to cope with stressful and adverse situations is different (Monclús et al., 2006; Cabezas et al., 2007; Broom, 2011).

Taking into account the large number of infrared temperature measurements recorded (456), it is safe to conclude that eye temperature is a good reference point to record body temperature in fattening rabbits by infrared thermography, since the range of temperature values recorded was the narrowest of all those taken, between 33.33 and 40.03 °C. However, no studies have correlated rectal temperature with eye temperature by thermography in rabbits, and further trials would therefore be needed to confirm this conclusion.

The highest temperature ranges in undisturbed rabbits occurred in the outer ear measurements, as reported by Gonzalez et al. (1971) and Zeferino et al. (2011), since this external body part is involved in heat transfer to the room, and is affected by both vasconstriction and vasodilatation, depending on a lower or higher room temperature, respectively (Cervera & Fernández-Carmona, 1998).

The range of differences between the infrared temperature averages for the outer ear and room temperature in undisturbed rabbits, considering both summer and winter, was 3.5, compared to 4.5 °C found by Gonzalez et al. (1971). However, if only infrared temperature values in summer are considered, the difference is 5.0 °C in our trial against 3.8 °C in Gonzalez et al. (1971) and 2.9 °C in Yamasaki-Maza et al. (2017). These differences may be due to factors such as differences in rabbit breeds and the tools used to measure temperature (Gonzalez et al., 1971). Yamasaki-Maza et al. (2017) used a clinical, digital non-contact infrared thermometer with temperature range of 32-43 °C (±0.3 °C accuracy) on New Zealand, Chinchilla and Azteca rabbits, while Gonzalez et al. (1971) used copper-constantan thermocouples attached with plastic discs to small shaved areas of skin behind the ears of New Zealand White rabbits.

There were no differences between sexes in temperature values in handled and undisturbed rabbits. The fact that these differences did not occur is due to the fact that during the study, the rabbits had not yet reached puberty (Lebas et al., 1997) being between 28 and 66 days old, with a fattening period of 38 days. Monclús et al. (2006) detected differences between sexes when rabbits were subjected to stress due to differences in the metabolism of glucocorticoids between males and females. Touma et al. (2003) also showed that there were differences in corticosterone metabolism between male and female rats. Another reason why there were no differences in temperatures between males and females after handling could be the fact that the stress that occurred was acute, caused by a short reaction, and influenced by an increase in catecholamines instead of glucocorticoids.

In conclusion, rabbits that have not been handled by humans during the lactation period do not become accustomed to handling in the fattening phase and stress occurs, as evidenced by the body temperature variations. Infrared thermography is a good technique for assessing by temperature records the acute stress of fattening rabbits as a result of handling, and the inner ear and the eye are the most reliable points to measure it.

References

Ardiaca M, Brotóns NJ, Montesinos A, 2010. Aproximación a las urgencias y cuidados intensivos en conejos, psitácidos y reptiles. Clínica Veterinaria de Pequeños Animales 30 (1): 5-14.

Bartolomé E, Sánchez MJ, Molina A, Schaefer AL, Cervantes I, Valera M, 2013. Using eye temperature and heart rate for stress assessment in young horses competing in jumping competitions and its possible influence on sport performance. Animal 7 (12): 2044-2053. https://doi.org/10.1017/S1751731113001626

Benato L, Rooney NJ, Murrell JC, 2019. Pain and analgesia in pet rabbits within the veterinary environment: a review. Vet Anaesth Analg 46 (2): 151-162. https://doi.org/10.1016/j.vaa.2018.10.007

Bilkó Á, Altbäcker V, 2000. Regular handing early in the nursing period eliminates fear responses toward human beings in wild and domestic rabbits. Dev Psychobiol 36 (1): 78-87. https://doi.org/10.1002/(SICI)1098-2302(200001)36:1<78::AID-DEV8>3.0.CO;2-5

BOE, 2013. Real Decreto 53/2013, de 1 de febrero, por el que se establecen las normas básicas aplicables para la protección de los animales utilizados en experimentación y otros fines científicos, incluyendo la docencia. Ministerio de la Presidencia, Gobierno de España. Boletín Oficial del Estado 34: 11370-11421.

Bowler K, Manning R, 1994. Membranes as the critical targets in cellular heat injury and resistance adaptation. In: Temperature adaptation of biological membranes; Cossins AR (ed.). Section 15, pp: 185-204. Portland Press, London. ISBN: 9781855780620.

Broom DM, 2011. Animal welfare: concepts, study methods and indicators. Rev Colomb Cienc Pec 24 (3): 306-321.

Cabezas S, Blas J, Marchant TA, Moreno S, 2007. Physiological stress levels predict survival probabilities...
in wild rabbits. Horm Behav 51 (3): 313-320. https://doi.org/10.1016/j.yhbeh.2006.11.004

Canón J, (Ed). 2015. Historia, caracterización y situación del conejo Antiguo Pardo Español. ASEMUCE-Servicio de Genética UCM. https://www.ucm.es/data/cont/docs/345-2016-12-07-Raza_Conejos_Antiguo_Pardo_Español.pdf.

Cervera C, Fernández-Carmona J, 1998. Nutrition and the climatic environment. In: The nutrition of the rabbit; De Blas C, Wiseman J (eds.). pp: 267-284. CAB Int, Wallingford, UK. ISBN: 978-0851992792. https://doi.org/10.1079/9781845936693.0267

Collier RJ, Baumgard LH, Zimmelbar RB, Xiao Y, 2019. Heat stress: physiology of acclimation and adaptation. Anim Front 9 (1): 12-19. https://doi.org/10.1093/af/apl031

Coureaud G, Rödel HG, Le Normand B, Fortun-Lamothe L, Bignon L, 2015. Habitat e comportement. In: Le lapin. De la biologie à l'élevage; Gidenne T (ed.). pp: 107-136. Éditions Quae, Versailles, France. ISBN: 9782759224166.

Csatádi K, Kustos K, Eiben C, Bilkó Á, Altbäcker V, 2005. Even minimal human contact linked to nursing reduces fear responses toward humans in rabbits. Appl Anim Behav Sci 95 (1-2): 123-128. https://doi.org/10.1016/j.applanim.2005.05.002

Daader AH, Al-Sagheer AA, Gabr HA, Abd El-Mo niem EA, 2018. Alleviation of heat-stress-related physiological perturbations in growing rabbits using natural antioxidants. Span J Agric Res 16 (3): e0610. https://doi.org/10.1052/j.sjar/2018163-13184

Dalmau A, Catanese B, Rafel O, Rodríguez P, Fuentes C, Llonch P, Mainain E, Velarde A, Ramón J, Taberner E, López-Béjar M, Piles M, 2015. Effect of high temperatures on breeding rabbit behaviour. Anim Prod Sci 55 (9): 1207-1214. https://doi.org/10.1071/AN13440

De Lima V, Piles M, Rafel O, López-Béjar M, Ramón J, Velarde A, Dalmau A, 2013. Use of infrared thermography to assess the influence of high environmental temperature on rabbits. Res Vet Sci 95 (2): 802-810. https://doi.org/10.1016/j.rvsc.2013.04.012

Dickens MJ, Romero LM, 2013. A consensus endocrine profile for chronically stressed wild animals does not exist. Gen Comp Endocr 191: 177-189. https://doi.org/10.1016/j.ygece.2013.06.014

Duval F, González F, Rabía H, 2010. Neurobiología del estrés. Revista Chilena de Neuro-Psiquiatría 48 (4): 307-318. https://doi.org/10.4067/S0717-92272010005000006

Emam AM, Afonso S, Azoz AAA, González-Redondo P, Mehaisen GMK, Ahmed NA, Ferrand N, 2016a. Microsatellite polymorphism in some Egyptian and Spanish common rabbit breeds. Proc. 11th World Rabbit Congress, Qingdao (China), Jun 15-18. pp: 31-34. Emam AM, Afonso S, Azoz AAA, González-Redondo P, Mehaisen GMK, Ahmed NA, Ferrand N, 2016b. Origin of Egyptian and Spanish common rabbits: evidence from mitochondrial DNA cytochrome b sequence analysis. Proc. 11th World Rabbit Congress, Qingdao (China), Jun 15-18. pp: 35-38.

FAOSTAT. 2019. Statistics Database. Food and Agriculture Organization of the United Nations. Rome (Italy). http://www.fao.org/faostat/en/#data

Fayez I, Marai M, Alnaimy A, Habeeb M, 1994. Thermoregulation in rabbits. In: Rabbit production in hot climates; Baselga M, Marai IFM (eds.), CIHEAM, Zaragoza. Cahiers Opt Méditerr 8: 33-41.

Ferré JS, Rosell JM 2000. Alojamiento y patología. In: Enfermedades del conejo; Rosell JM (ed.). Vol. II, pp: 167-210. Mundi-Prensa, Madrid. ISBN: 9788471149077.

Ferreira C, Castro F, Piorno V, Catalán I, Delibes-Mateos M, Rouco C, Mínguez LE, Aparicio F, Blanco-Aguiria JA, Ramírez E, et al., 2015. Biometrical analysis reveals major differences between the two subspecies of the European rabbit. Biol J Linn Soc 116 (1): 106-116. https://doi.org/10.1111/bij.12556

Gascón FM, Verde M, 1987. Efecto estresante de la manipulación en el conejo. Proc. XII Simp. de Cunicultura de ASECUCU, Guadalajara (Spain), May 20-22. pp: 125-132.

Glaser R, Kiecolt-Glaser JK, 2005. Stress-induced immune dysfunction: implications for health. Nat Rev Immunol 5(3): 243-251. https://doi.org/10.1038/nri1571

Gonzalez RR, Kluger MJ, Hardy JD, 1971. Partially calorimetry of the New Zealand White rabbit at temperatures 5-35 °C. J Appl Physiol 31 (5): 728-734. https://doi.org/10.1152/jappl.1971.31.5.728

González-Redondo P, 2016. Resultados preliminares de rendimiento reproductivo y de engorde de un núcleo de cría de conejos de tipo Común Doméstico Español. Proc. XLI Simp. de Cunicultura de ASECUCU, Hondarribia (Spain), May 12-13. pp: 180-185.

Greco D, Stabenfeldt GH, 1997. Endocrine glands and their function. In: Textbook of veterinary physiology; Cunningham JG (ed.). 2nd ed, pp: 404-439. WB Saunders Company St. Louis, USA. ISBN: 9780721664248.

Grissom N, Bhattacharjee BC, 2009. Habituation to repeated stress: get used to it. Neurobiol Learn Mem 92 (2): 215-224. https://doi.org/10.1016/j.nlm.2008.07.001

Kataoka N, Hikoi H, Kaneko T, Nakamura K, 2014. Psychological stress activates a dorsomedial hypothalamus-medullary raphe circuit driving brown

Spanish Journal of Agricultural Research June 2020 • Volume 18 • Issue 2 • e0502
adipose tissue thermogenesis and hyperthermia. Cell Metab 20 (2): 346-358. https://doi.org/10.1016/j.cmet.2014.05.018

Kuchel O, 1991. Stress and catecholamines. Methods Achiev Exp Pathol 14: 80-103.

Lattin CR, Romero LM, 2014. Chronic stress alters concentration of corticosterone receptors in a tissue-specific manner in wild house sparrows (Passer domesticus). J Exp Biol 217 (14): 2601-2608. https://doi.org/10.1242/jeb.103788

Lebas F, Coudert P, de Rochambeau H, Thébault RG, 1997. The rabbit - Husbandry, health and production. FAO, Rome. ISBN: 9789251034415.

Long NC, Vander AJ, Kluger MJ, 1990. Stress-induced rise of body temperature in rats is the same in warm and cool environments. Physiol Behav 47 (4): 773-775. https://doi.org/10.1016/0031-9384(90)90093-J

Ludwig N, Gargano M, Luzi F, Carenzi C, Verga M, 2007. Technical note: applicability of infrared thermography as a non invasive measurement of stress in rabbit. World Rabbit Sci 15 (4): 199-206. https://doi.org/10.4995/wrs.2007.588

Luzi F, Ludwig N, Monzani M, Gargano M, Ricci C, Redaelli V, Verga M, 2007. Procedures for analyses of sequence of thermal images in welfare study of rabbit. Proc. 4th Int. Workshop on the Assessment of Animal Welfare at Farm and Group Level (WAFL), Ghent (Belgium), Sep 10-13. p. 85.

Manteca X, 1998. Neurophysiology and assessment of welfare. Meat Sci 49 (Suppl 1): S205-S218. https://doi.org/10.1016/S0309-1740(98)90049-3

Minton JE, 1994. Function of the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system in models of acute stress in domestic farm animals. J Anim Sci 72 (7): 1891-1898. https://doi.org/10.2527/1994.7271891x

Monclús R, Rödel HG, Palme R, von Holst D, De Miguel J, 2006. Non-invasive measurement of the physiological stress response of wild rabbits to the odour of a predator. Chemoecology 16 (1): 25-29. https://doi.org/10.1007/s00049-005-0324-6

Möstl E, Palme R, 2002. Hormones as indicators of stress. Domest Anim Endocrin 23 (1-2): 67-74. https://doi.org/10.1016/S0739-7240(02)00146-7

Nelson RJ, 2000. An introduction to behavioral endocrinology, 2nd edition. Sinauer Ass. Inc. Pub., MA, USA. ISBN: 0-87893-616-5

OJ, 2010. Directive 2010/63/EU of 22 September 2010 on the protection of animals used for scientific purposes. European Parliament and Council. Official J European Union, L276: 33-79.

Okab AB, El-Banna SG, Koriem AA, 2008. Influence of environmental temperatures on some physiological and biochemical parameters of New-Zealand rabbit males. Slovak J Anim Sci 41 (1): 12-19.

Olivas I, Villagrá A, 2013. Technical Note: Effect of handling on stress-induced hyperthermia in adult rabbits. World Rabbit Sci 21 (1): 41-44. https://doi.org/10.4995/wrs.2013.1178

Peaston RT, Weinikove C, 2004. Measurement of catecholamines and their metabolites. Ann Clin Biochem 41 (1): 17-38. https://doi.org/10.1258/000456304432266463

Podberscek AL, Blackshaw JK, Beattie AW, 1991. The behaviour of group penned and individually caged laboratory rabbits. Appl Anim Behav Sci 28 (4): 353-363. https://doi.org/10.1016/0168-1591(91)90167-V

Price EO, 2002. Animal domestication and behavior. CAB Int, Wallingford, UK. ISBN: 9780851995977. https://doi.org/10.1079/9780851995977.0000

Samoggia G, 1987. Esigenze fisioclimatiche dei conigli nell'allevamento intensivo. Rivista di Coniglicoltura 24 (5): 16-20.

Sánchez MJ, Bartolomé E, Valera M, 2016. Genetic study of stress assessed with infrared thermography during dressage competitions in the Pura Raza Español horse. Appl Anim Behav Sci 174: 58-65. https://doi.org/10.1016/j.applanim.2015.11.006

Samniguel RA, Díaz V, 2011. Mecanismos fisiológicos de la termorregulación en animales de producción. Rev Colombl Cienc Anim 4 (1): 88-94.

Silva S, Mourão JL, Ribeiro L, Gonçalves C, Pinheiro V, 2014. Utilização de imagens termográficas por infravermelhos para avaliar a temperatura de láparos em ninhos com diferente material. Proc. XXXIX Simp. de Cunicultura de ASESCU, Tudela (Spain), May 29-30. pp: 67-70.

Silva SR, Mourão JL, Guedes C, Monteiro D, Pinheiro V, 2015. Utilização de imagens video e termográficas para estimar o peso vivo de conejos em cebo. Proc. XL Simp. de Cunicultura de ASESCU, Santiago de Compostela (Spain), May 28-29. pp: 115-117.

Soleimani AF, Kasiim A, Alimon AR, Zulkifli I, 2008. Durability of induced heat tolerance by short term heat challenge at broilers marketing age. Pak J Biol Sci 11 (27): 2163-2166. https://doi.org/10.3923/pjbs.2008.2163.2166

Stewart M, Webster JR, Schaefer AL, Cook NJ, Scott SL, 2005. Infrared thermography as a non-invasive tool to study animal welfare. Anim Welfare 14 (4): 319-325.

Stewart M, Webster JR, Verkerk GA, Schaefer AL, Colyn JJ, Stafford KJ, 2007. Non-invasive measurement of stress in dairy cows using infrared thermography. Physiol Behav 92 (3): 520-525. https://doi.org/10.1016/j.physbeh.2007.04.034

Temple D, Mainau E, Manteca X, 2014. Practical note – Fear caused by poor human-animal relationship. Farm Animal Welfare Education Centre. https://www.fawec.org/media/com_lazypdf/pdf/Nota-n-2-fear-en.pdf
Touma C, Sachser N, Möstl E, Palme R, 2003. Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. Gen Comp Endocrinol 130 (3): 267-278. https://doi.org/10.1016/S0016-6480(02)00620-2

Travain T, Colombo ES, Heinzl E, Bellucci D, Previde EP, Valsecchi P, 2015. Hot dogs: Thermography in the assessment of stress in dogs (Canis familiaris)-A pilot study. J Vet Behav 10 (1): 17-23. https://doi.org/10.1016/j.jveb.2014.11.003

Trocino A, Xiccato G, 2006. Animal welfare in reared rabbits: a review with emphasis on housing systems. World Rabbit Sci 14 (2): 77-93. https://doi.org/10.4995/wrs.2006.553

Valera M, Bartolomé E, Sánchez MJ, Molina A, Cook N, Schaefer AL, 2012. Changes in eye temperature and stress assessment in horses during show jumping competitions. J Equine Vet Sci 32 (12): 827-830. https://doi.org/10.1016/j.jevs.2012.03.005

Veissier I, Boissy A, 2007. Stress and welfare: Two complementary concepts that are intrinsically related to the animal's point of view. Physiol Behav 92 (3): 429-433. https://doi.org/10.1016/j.physbeh.2006.11.008

Warriss PD, Pope SJ, Brown SN, Wilkens LJ, Knowles TG, 2006. Estimating the body temperature of groups of pigs by thermal imaging. Vet Rec 158 (10): 331-334. https://doi.org/10.1136/vr.158.10.331

Wingfield JC, Hunt K, Breuner C, Dunlap K, Fowler GS, Freed L, Lepson J, 1997. Environmental stress, field endocrinology, and conservation biology. In: Behavioral approaches to conservation in the wild; Buchholz JR, Clemmens R (eds.). pp: 95-131. Cambridge University Press, Cambridge, UK. ISBN: 9780521589604.

Xu HT, 1996. The behaviour of the rabbit. Proc. 6th World Rabbit Congress. Toulouse (France), Jul 09-12. pp: 437-440.

Yamasaki-Maza A, Yamasaki-Maza L, Ruiz-Rojas JL, 2017. Temperatura ambiente y humedad relativa y su relación con el bienestar en conejos (Oryctolagus cuniculus) en engorda en el trópico seco. Proc. Congr. Mesoam. de Invest. UNACH. Digital Magazine 4: 1366-1371.

Zeferino CP, Moura ASAMT, Fernandes S, Kanayama JS, Scapinello C, Sartori JR, 2011. Genetic group × ambient temperature interaction effects on physiological responses and growth performance of rabbits. Livest Sci 140 (1-3): 177-183. https://doi.org/10.1016/j.livsci.2011.03.027

Zucca D, Redaelli V, Marelli SP, Bonazza V, Heinzl E, Verga M, Luzi F, 2012. Effect of handling in pre-weaning rabbits. World Rabbit Sci 20 (2): 97-101. https://doi.org/10.4995/wrs.2012.1083