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RESEARCH ARTICLE

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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 ºC for eye to 3.09±0.221 ºC for outer ear in summer and -0.41±0.182 ºC for nose to 2.09±0.178 ºC 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 ºC *vs.* 0.54 to 5.52 ºC, 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).

Authors' contributions: Conceived and designed the experiments, and wrote the paper: JAJT, MV, MJSG and PGR. Performed the experiments and acquired the data: JAJT, JILC and MJSG. Analysed the data: MJSG and MV. All the authors revised and approved the final manuscript.

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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 sympatho-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 ºC for the fattening phase (Ferré & Rosell, 2000; Coureaud *et al.*, 2015), and 16-20 ºC 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; Csatádi *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 handling 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.

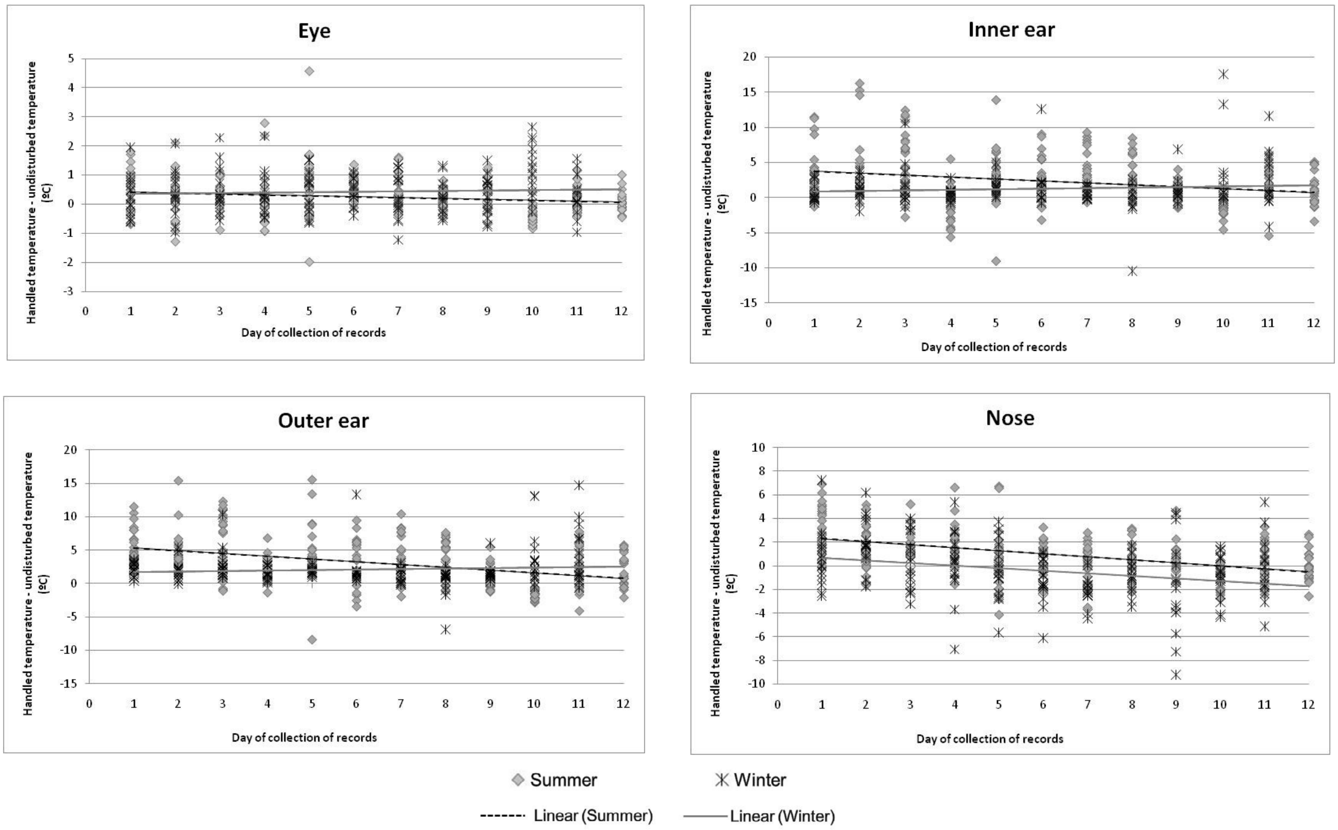

Figure 1. Evolution of the variation in temperatures (ºC) between Infrared Temperature in handled rabbits and in undisturbed rabbits for eye, inner ear, outer ear and nose during summer and winter (12 days of sampling per season).

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 and 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.	***	*
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.	*	**	**
Inner ear VAR	n.s.	***	n.s.	n.s.	n.s.	n.s.
Outer ear VAR	n.s.	***	n.s.	n.s.	***	n.s.
Nose VAR	n.s.	***	*	n.s.	***	n.s.
DIF Eye-TEMP	n.s.	***	n.s.	n.s.	***	n.s.
DIF Inner ear-TEMP	n.s.	***	n.s.	n.s.	***	n.s.
DIF Outer ear-TEMP	n.s.	***	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)			
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}
			Winter (n = 187)			
Eye	36.83^{aA}	37.05^{aA}	0.21	37.35^{bA}	37.58^{bA}	0.23
Inner ear	16.80^{aA}	17.67^{aA}	0.86^{A}	20.53^{bA}	22.65^{bA}	2.11
Outer ear	14.69^{aA}	16.91^{aA}	2.21^{A}	18.50^{bA}	22.37^{bA}	3.87
Nose	29.86^{aA}	30.48^{aA}	0.61^{A}	33.32^{bA}	32.95^{bA}	-0.37

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 rabbits and inner ear temperature in handled animals) to 0.95 (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 (Gascón & 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). Rabbits 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 ºC in summer and 39.75 ºC in the outer ear in winter; as a result, hyperthermia by induction of stress was not pathological for the

Table 5. Pearson correlation among all the traits studied (temperature in ºC 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*	0.53*	-0.63*	-0.26*
Eye, H	0.95*		0.62*	0.68*	0.63*	0.68*	0.58*	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. *: $p<0.05$

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. Continued

	Outer ear VAR	Nose VAR	DIF Eye-TEMP	DIF Inner ear-TEMP	DIF Outer ear-TEMP	DIF Nose-TEMP	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-TEMP	0.05	-0.22*		0.96*	0.66*	0.51*	-0.45*	-0.37*	0.37*	-0.41*
DIF Inner ear-TEMP	-0.05	-0.26*	0.83*		0.62*	0.44*	-0.38*	-0.44*	0.45*	-0.47*
DIF Outer ear-TEMP	-0.05	-0.67*	0.36*	0.45*		0.85*	-0.81*	0.10	-0.11	0.02
DIF Nose-TEMP	-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-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 ºC, 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 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 ºC. 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 vasoconstriction 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 ºC found by Gonzalez *et al*. (1971). However, if only infrared temperature values in summer are considered, the difference is 5.0 ºC in our trial against 3.8 ºC in Gonzalez *et al*. (1971) and 2.9 ºC 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 ºC (±0.3 ºC 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.

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