

RELATIONSHIP BETWEEN RECTAL TEMPERATURE MEASURED WITH A CONVENTIONAL THERMOMETER AND THE TEMPERATURE OF SEVERAL BODY REGIONS MEASURED BY INFRARED THERMOGRAPHY IN FATTENING RABBITS. INFLUENCE OF DIFFERENT ENVIRONMENTAL FACTORS

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Abstract: In clinical examination of rabbits, the temperature is usually recorded with a digital thermometer introduced rectally, an invasive procedure that could cause handling stress. The aim of this study was to assess body temperature using infrared thermography (IRT) in four areas of the rabbit's anatomy: eye (ETT), outer ear (OETT), inner ear (IETT) and nose (NTT), and then validate it as an alternative measure to rectal temperature (RT) assessed with a conventional thermometer. Temperature samples were taken twice a week from 48 weaned rabbits of Spanish Common Rabbit breed during a 38-d fattening period. The factors considered were: doe from which the rabbits came (8 does), weeks of fattening period (4 to 5 wk), batch (3 periods of the year: April-May, June-July and January-February) and group size (cages with 1 to 7 rabbits). On average, the results were an RT of 38.48 ± 0.02 °C; ETT of 37.31 ± 0.05 °C; OETT of 29.09 ± 0.26 °C; IETT of 30.53 ± 0.25 °C, and NTT of 33.29 ± 0.11 °C (mean \pm se). Moderate, statistically significant positive correlations ($P < 0.001$) were observed between RT and temperatures measured with infrared thermography (IETT, OETT, ETT and NTT), both in general (0.39 to 0.49) and intraclass (0.36 to 0.39), based on the batch, group size, week of fattening period and doe. The thermographic measurements which showed the highest correlation with RT were OETT and IETT. We also studied the effects that could influence the temperature variables evaluated by IRT and RT within each batch: for each week of the fattening period, for the group size and for the doe effect. We found significant differences ($P < 0.001$) between weeks within the batch, with a tendency for the temperature of the rabbits to increase as the fattening period progressed. The doe effect (within the batch) did not show, on the whole, any statistically significant differences within batches. On the other hand, we did observe a trend towards higher temperatures as the group size increased. In conclusion, infrared thermography is an effective tool for body temperature assessment and correlates closely with RT, with IETT appearing as the best reference point for taking body temperature in fattening rabbits. Infrared thermography appeared as a suitable alternative to RT for body temperature assessment in rabbits, thus avoiding handling stress.

Key Words: rectal temperature, infrared thermography, rabbit, fattening period, group size, season.

INTRODUCTION

The infrared thermography (IRT) technique, using an infrared thermography camera, works by measuring electromagnetic energy and detecting the different wavelengths emitted by each temperature value (Stewart *et al.*, 2005). Its main strength is that it is a rapid, non-invasive, non-contact method to measure the animal's surface temperature (De Lima *et al.*, 2013; McCafferty, 2013). It allows us to evaluate the physiological states and temperature changes due to pathologies and to estimate the live weight of the fattening rabbits (Silva *et al.*, 2015a; Travain *et al.*, 2015; Sánchez *et al.*, 2016). However, it may be conditioned by certain factors such as climate conditions (Knizková *et al.*, 2007).

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Rabbits are quiet and docile animals, although they tend to be skittish (Ward, 2006). Their relatively recent domestication means that they continue to see humans as predators, so they instinctively associate their presence with negative stimuli, which constitutes a major factor of stress and fear (Trocino and Xiccato, 2006). In this way, handling rabbits wrongly can have physical (spinal cord or vertebrae fracture or subluxation) and physiological (hypertension, hyperglycaemia, decreased renal perfusion, intestinal hypomotility) consequences on the animals due to stress, and can lead to various types of pathologies, such as diarrhoea (Lebas *et al.*, 1997; Meredith, 2006; Malley, 2007), producing weight loss and even death (Lebas *et al.*, 1997).

The rabbit is a homeothermic animal, so it has to keep its body temperature constant in order to carry out its normal activity and vital processes (Yamasaki-Maza *et al.*, 2017). Body temperature is a physiological parameter that allows us to measure the animals' health status (Valera *et al.*, 2012), as most infectious diseases present with hyperthermia (Zimmerman *et al.*, 2012), as well as the rabbit's welfare by assessing its stress level, as the higher the stress, the lower the welfare (Stewart *et al.*, 2005). Stress causes the release of various hormones in rabbits, such as catecholamines (especially adrenaline and norepinephrine), corticotropin-releasing hormone, adrenocorticotrophic hormone and corticosteroids (in rabbits, mainly corticosterone and cortisol), which cause an increase in body temperature (Kataoka *et al.*, 2014), among other effects.

There are various devices available to help us measure an animal's body temperature, both internally and externally (Flecknell, 2009). The most widely-used technique in rabbits is recording the rectal temperature (RT) (Chen and White, 2006), which is obtained by inserting a conventional thermometer into the rectum (Chapel *et al.*, 2015). Other alternative options which have been tested and validated include taking the temperature either in the tympanic membrane with an infrared thermometer (Hall *et al.*, 2001; Ardiaca *et al.*, 2010) or on the surface of the skin using IRT (Chacur *et al.*, 2016). In rabbits, different anatomical parts present different temperature values due to different vascularisation and the influence of environmental temperature (Jaén-Téllez *et al.*, 2020).

Changes in body temperature can be measured correctly using RT (Marai *et al.*, 2002), but this methodology is invasive and could negatively affect the animal's welfare, as it takes a long time to measure (Chung *et al.*, 2010; Vicente-Pérez *et al.*, 2019).

However, no previous recorded studies have compared the rabbit's body temperature using RT with IRT measurements taken in different body regions on a routine basis. Therefore, the main aim of this study was to evaluate the temperature taken with an IRT camera in four different parts of the anatomy in fattening rabbits (eye, outer ear, inner ear and nose), in order to validate it as an alternative to RT using a conventional thermometer, and to assess the influence of different environmental effects (doe, week of the fattening period and group size) on this evaluation.

MATERIALS AND METHODS

Animals and husbandry

We analysed a total of 48 rabbits from 8 different does (Figure 1), weaned at 28 d of age, over a fattening period of 38 d. All the rabbits analysed were from the common Spanish agouti-coated domestic meat-oriented breed, belonging to a strain kept at the Higher Technical School of Agricultural Engineering Teaching Farm of the University of Seville (Spain). The genetic characterisation and productive performance of this stock have previously been described by Emam *et al.* (2020) and González-Redondo (2016), respectively. Overall, the rabbits were phenotypically similar to the recently recognised autochthonous breed "Antiguo Pardo Español" (Spanish Common Rabbit) (Ministerio de Agricultura, Pesca y Alimentación, 2019).

During three experimental periods (batches), the rabbits were housed in polyvalent wire-mesh cages measuring 90×40×30 cm (length, width and height, respectively), located in a conventional closed facility with natural (non-forced) ventilation, with geographic coordinates: 37° 21' 36.3" N and 5° 56' 23.9" W; 11 m a.s.l., and the animals were subjected to a natural photoperiod. The experiment was carried out in accordance with the Royal Decree 53/2013 (Ministerio de la Presidencia, 2013) and the Directive 2010/63/EU on the protection of animals used for scientific purposes (EU Parliament and Council, 2010).

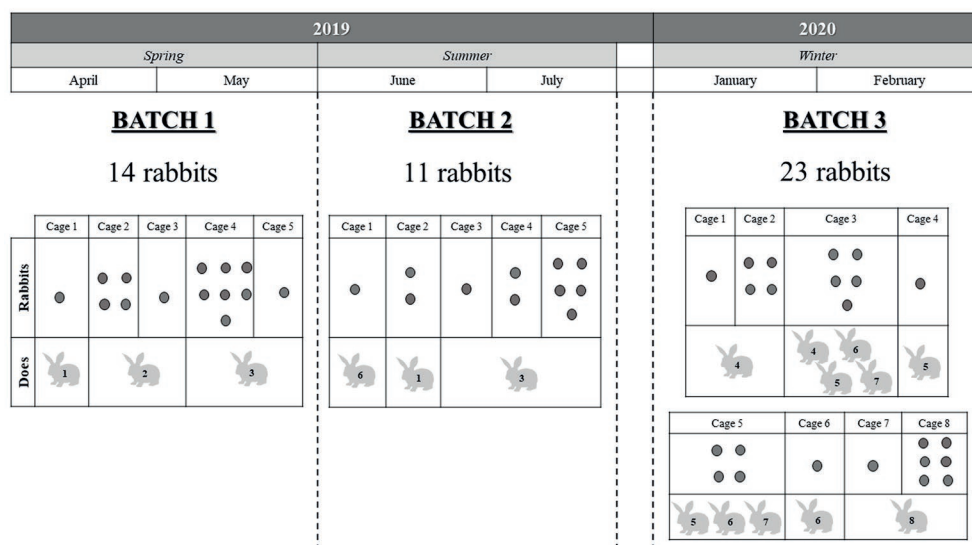


Figure 1: Distribution of animals within batches, cages and does.

The rabbits were fed a commercial pelleted balanced diet (15.0% crude protein and 15.5% crude fibre) *ad libitum*. Water was supplied *ad libitum*. No antibiotics were added to the water or food.

Temperature recording

Infrared thermography images were taken with a FLIR E60 camera, following the indications by Bartolomé *et al.* (2013) and Jaén-Téllez *et al.* (2020). To calibrate the camera results, the environmental temperature and relative humidity were recorded with a digital thermo-hygrometer (Extech® 44550) every time an animal was measured, so that each infrared temperature had corresponding humidity and temperature values from the room where the trial took place. Later, these thermographic photos were analysed with Flir Tools+® software, obtaining for the analyses the maximum temperature (indicated with a red triangle) of a circle traced around the body area evaluated (Figure 2).

Rectal temperature was measured using a conventional clinical thermometer (Thermoval® kids flex), following the technical indications from Chapel *et al.* (2015). To do this, the rabbit was held in sternal decubitus, while the tail was moved to one side and a lubricated thermometer was inserted through the anus into the rectum, 60 s after taking the rabbit out of the cage. To avoid the possibility of spreading diseases between the animals, the thermometer was disinfected with alcohol after measuring each rabbit's temperature.

Experimental procedure

The sample temperatures were collected twice a week (Monday and Thursday), between 11:00 a.m. to 12:00 p.m., during the fattening period (38 d). In order to test the influence of different factors on the temperature of the rabbits, 4 different effects were studied: doe (doe 1 to doe 8), batch (batch 1 to batch 3), week of fattening period (week 1 to week 5) and group size (individual, small group and large group).

The criteria for forming the groups according to "group size" were having individual animals, small groups of rabbits and large groups of rabbits. Individual groups contained just 1 rabbit, small groups contained 2 to 4 rabbits and large groups contained 5 or more.

The three seasonal periods or batches of the trial were arranged as follows: batch 1 (Spring: April-May 2019), batch 2 (Summer: June-July 2019) and batch 3 (Winter: January-February 2020). Batch 1 included 14 animals from

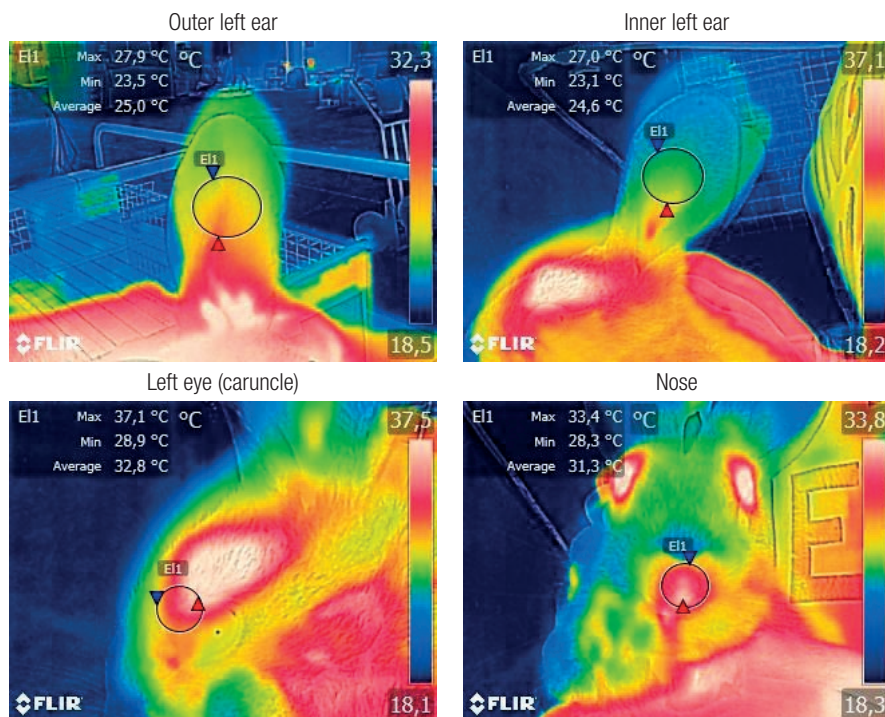


Figure 2: Thermographic images of different body regions of the rabbit.

E1 identifies the circle traced around the measured part of the body of the rabbit; the red triangle indicates the maximum temperature point within the E1 circle; the blue triangle indicates the minimum temperature point within the E1 circle; the rectangle on the upper left side of the photo indicates the values measured within the E1 circle (maximum, minimum and average temperature, in °C); the coloured line along the right side of the photo indicates the temperature colour range of the whole photo, with the minimum temperature in the bottom blue side and the maximum temperature at the top white side (in °C).

3 different does, which were housed in 5 different cages: 1 small group cage, 1 large group cage and 3 individual cages. During batch 2, 11 animals were measured, from 3 different does and housed in 5 different cages: 2 small group cages, 1 large group cage and 2 individual cages. Finally, 23 animals were measured during batch 3, from 5 different does, which were housed in 8 different cages: 2 small group cages, 2 large group cage and 4 individual cages (Figure 1).

During each sampling session, two infrared thermography photos were taken from 4 different anatomical points on each rabbit (8 infrared thermography photos per animal and session in total): outer and inner parts of the left ear, left eye caruncle and whole nose (Figure 2). The photos were always taken in the same order for all the animals, and it took 30 s approximately per animal in all cases. In addition, a rectal temperature measurement was also taken just after the infrared thermography photos were obtained. The approximate total time per animal for taking thermographic photographs and measuring the rectal temperature was 1 min 30 s. After all the samples were obtained, the animal was put back in its cage and the next rabbit was taken for measuring. Thus, a total of 9 temperature measurements per animal and session were obtained. Taking into consideration that the samples were taken twice a week for 38 d (about 8 measuring sessions per fattening period), a total of 72 temperature measurements were obtained per animal. As this process was repeated each seasonal period, 417 temperature results were obtained in total from 48 different rabbits: 126 in batch 1 (Spring), 88 in batch 2 (Summer) and 203 during batch 3 (Winter).

Statistical analyses

A previous Shapiro-Wilk test (results not shown) presented a normal distribution of the variables studied. Hence, parametric statistical analyses were carried out.

First of all, a descriptive statistical analysis (mean, standard error, standard deviation, minimum and maximum) was performed on the 48 rabbits studied during the fattening period for the five variables analysed: rectal temperature (RT), eye thermographic temperature (ETT), inner ear thermographic temperature (IETT), outer ear thermographic temperature (OETT) and nose thermographic temperature (NTT). In addition, an independent *t*-test by variable was carried out to search for statistically significant ($P<0.05$) differences between the rectal (RT) and thermographic (ETT, IETT, OETT and NTT) variables.

To obtain the phenotypic correlations between the rectal and thermographic variables, direct and intraclass Pearson's correlations, according to batch, group size, week of the fattening period and doe, were computed. Scatter plots and regression analyses between rectal temperature (RT) and thermographic temperatures were also performed, and the coefficient of determination (R^2) and the root-mean-square error (RMSE) for them were calculated.

To test the influence of environmental factors on the rabbits' temperatures, a univariate General Linear Model (GLM) analysis was carried out for the five temperatures studied (RT, ETT, IETT, OETT and NTT) according to doe, group size and week of fattening period, all within each batch. Moreover, to determine any statistically significant ($P<0.05$) differences between the levels of the effects for the RT variable, a post-hoc Duncan's test was carried out.

Statistical analyses were performed using SPSS software for Windows (IBM Corp. IBM SPSS Statistics for Windows; Version 25.0; IBM Corp: Armonk, NY, USA, 2017).

RESULTS AND DISCUSSION

Measuring body temperature is a key task in the sanitary management of a livestock farm, especially in rabbits, since, being a prey animal, it tends to hide the symptoms of its state of health (Ardiaca *et al.*, 2010). Both hyperthermia and hypothermia are sure signs of health problems in animals. Hyperthermia can be caused by infection, stress, inflammations of non-infectious origin or unfavourable environmental conditions (Fraser, 1988), while hypothermia can be caused by unfavourable environments, the inability of the hypothalamus to control body temperature due to intracranial diseases (Fraser, 1988) or by a number of different pathologies, such as coccidiosis (Vadlejch *et al.*, 2010).

The highest mean temperature in the rabbits was found for RT (38.48 ± 0.02), followed by ETT (37.31 ± 0.05), NTT (33.29 ± 0.11), IETT (30.53 ± 0.25) and lastly OETT (29.09 ± 0.26) (Table 1). The temperatures taken by IRT were consistent with those obtained by Jaén-Téllez *et al.* (2020) in a similar study in rabbits of the same breed. The difference in temperature values for each anatomical point studied can be explained by different vascularisation and by the influence of the ambient temperature (Jaén-Téllez *et al.*, 2020). The average normal body temperature

Table 1: Descriptive statistics (mean, standard error, minimum, maximum, standard deviation) for the 4 thermographic variables and for rectal temperature. T-test by variable between the 4 thermographic temperature variables analysed and RT.

	Mean \pm s.e. (°C)	Min	Max	s.d.	P-value
RT	38.48 \pm 0.02	35.60	39.80	0.50	-
IETT	30.53 \pm 0.25	16.00	40.35	5.11	***
OETT	29.09 \pm 0.26	18.20	39.10	5.41	***
ETT	37.31 \pm 0.05	32.10	39.45	1.10	***
NTT	33.29 \pm 0.11	26.15	38.45	2.17	***

*** $P<0.001$; RT: Rectal temperature; ETT: Thermographic temperature from the eye; IETT: Thermographic temperature from the inner ear; OETT: Thermographic temperature from the external ear; NTT: Thermographic temperature from the nose, s.e.: standard error; s.d.: standard deviation.

of the rabbits taken by RT in this study ($38.5\pm 0.02^\circ\text{C}$) was found to be within the ranges established by various authors ($38.0\text{-}40.0^\circ\text{C}$ according to Harris, 1994; $37.7\text{-}39.0^\circ\text{C}$ according to Ardiaca *et al.*, 2010 and $38.0\text{-}38.5^\circ\text{C}$ according to Fuentes *et al.*, 2010). These temperature variations were due to different conditioning factors, such as environmental temperature, the rabbits' breed or the measuring instruments used (Zeferino *et al.*, 2011).

RT was higher than the temperatures measured by IRT in the different body areas analysed ($P<0.001$). The lowest temperature range could be observed in RT (35.6 to 39.8°C), which is an internal temperature, compared to the temperatures taken with IR, where the narrowest range was that of ETT (32.1 to 39.5°C), which coincides with the area with the highest temperature and with the data obtained by Jaén-Téllez *et al.* (2020).

All the correlations of IRT with RT (Figure 3) were positive, statistically significant ($P<0.001$) and of medium magnitude. The values ranged from 0.36 for the intraclass correlation with ETT and NTT to 0.49 for the general correlation with OETT.

Different authors have stated that RT correlates positively with IRT taken in different parts of the animal's anatomy. Vicente-Pérez *et al.* (2019) observed, in sheep, that RT is positively and moderately correlated with different hair coat temperatures obtained by IRT. Rydygier *et al.* (2017), working with buffaloes, concluded that the temperature of the

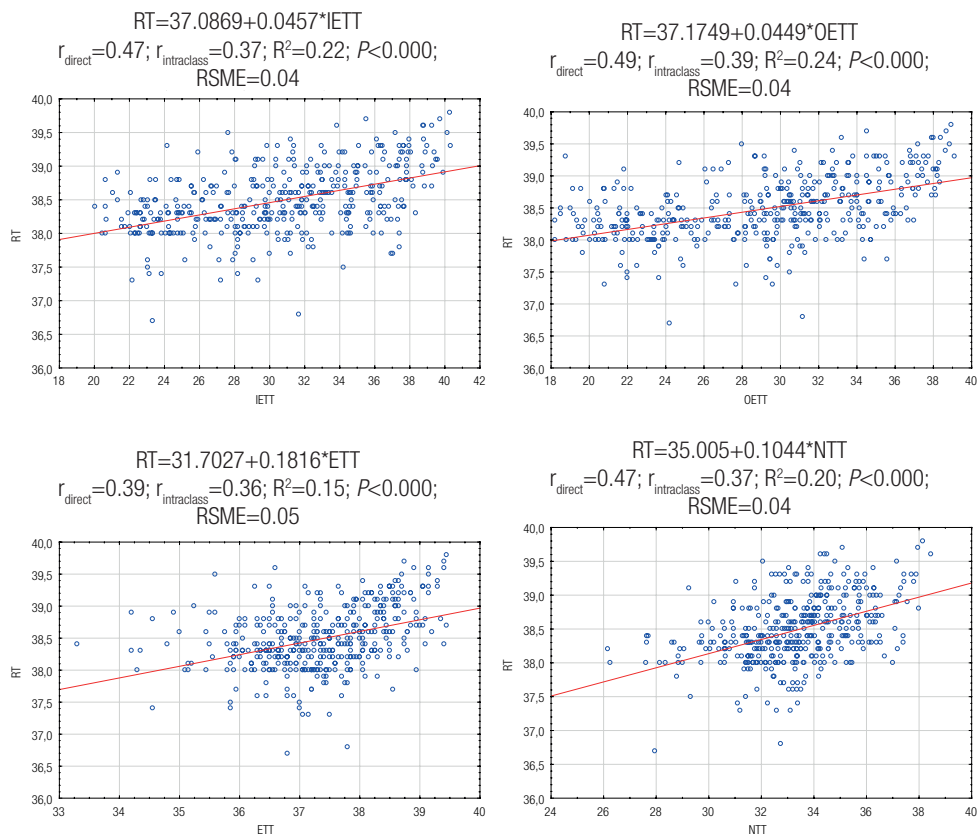


Figure 3: Pearson correlations (direct and intraclass according to batch, group size, week of fattening period and doe) and scatter plots with regression analysis between rectal temperature (RT) and thermographic temperatures.

r: Pearson correlation; R^2 : Coefficient of determination; RMSE: Root-mean-square error; RT: Rectal temperature; ETT: Thermographic temperature from the eye; IETT: Thermographic temperature from the inner ear; OETT: Thermographic temperature from the external ear; NTT: Thermographic temperature from the nose.

vulvar, nasal and ocular regions obtained by IRT correlated positively with RT. Chung *et al.* (2010) showed, in piglets, a linear relationship between RT and IRT taken in the central abdomen, cranial dorsum and perianal regions. Our study agrees with these authors, and shows the potential suitability of infrared thermography as a technique for measuring temperature in the same way as a digital thermometer.

In particular, the OETT and IETT variables showed the best correlations with RT, and their complementary use can therefore be recommended. In this context, De Lima *et al.* (2013) recommend taking the body temperature in rabbits using thermography in the eyes and nose due to the difficulty of taking it in the ear. Both the ears and the nose are used by rabbits to dissipate heat (Fayez *et al.*, 1994), although nose humidity can vary according to the ambient temperature in the different seasons, which can alter the value of the NTT (Luzi *et al.*, 2007). Jaén-Téllez *et al.* (2020) stated that ETT is the reference temperature for recording body temperature with thermography in fattening rabbits, as it is the anatomical area with the lowest range of variation. However, although the correlation results highlighted ear measurements (IETT and OETT) as better measurements to complement the RT measurement, IETT would be a better reference measure than OETT, as the outer ear is used by rabbits to dissipate heat (Fayez *et al.*, 1994).

Figure 4 shows the effect of the doe, week of fattening period and group size on the different temperature variables evaluated by IRT and RT, within each batch. For the doe effect, statistically significant differences were only found for the IETT and OETT variables of batch 1 and 3 and the RT of batch 2. Doe 3 showed statistically higher values ($P < 0.05$) in batch 1 and batch 2, as did doe 7 ($P < 0.05$) in batch 3. The differences between the does in specific batches and in certain anatomical parts could be explained by the individual effect of the doe. Quevedo *et al.* (2003) showed that the doe influences the productivity in the fattening area (growth and mortality during the fattening period), either by the number of parturitions the doe has or by the rabbit itself (individual effect).

As regards the batch-week of the fattening period combinations, we observed statistically significant differences ($P < 0.001$) between weeks for all the batches and for all the variables analysed (IRT and RT). The temperature increased with the weeks of the fattening period, showing an upward trend as the fattening period progressed. According to Fraser (1988), young animals tend to have lower temperatures than older animals. This has been observed not only in rabbits (Fewel *et al.*, 2000; Zeferino *et al.*, 2011; Daader *et al.*, 2018) but also in other species, such as broilers (Soleimani *et al.*, 2008) or dairy cattle (Collier *et al.* 2019).

In addition, different authors (Cervera and Fernández-Carmona, 1998; Jaén-Téllez *et al.*, 2020) noted that the temperature of the rabbit's different anatomical parts correlates with the environmental temperature. The environment has an influence on the surface temperature of the rabbit's body, because it affects skin temperature and the heat exchange between the animal and its environment through vasomotor control (Collier *et al.*, 2006; Zeferino *et al.*, 2011; Arduini *et al.*, 2017). A key factor in the regulation of a rabbit's internal temperature is therefore the environmental temperature at which the animals are kept (Sanmiguel and Díaz, 2011).

The animals' increased body temperature in each of the weeks of the fattening period may correlate with the evolution of the rising environmental temperature in each of the months of each batch. In order of the batches, the ambient temperature was higher at the end than at the beginning of each of these periods. The average environmental temperatures ranged from 21.3 °C in April to 38.7 °C in May for batch 1; 25.0 °C in June to 26.1 °C in July for batch 2, and 12.9 °C in January to 18.8 °C in February for batch 3.

For group size within each batch, we only observed statistically significant differences ($P < 0.05$) for the IETT and OETT variables of batch 1, with the animals in the small group showing the highest temperature values for both variables, compared to those in the large group. Stauffacher (1986 and 1992) recommended group housing in this species during the fattening period, as is common practice in industrial rabbit farming, since it increases their social contact and encourages development of the typical behaviours of the species. It also reduces biting the cage bars (a very common habit), aids thermoregulation and can help relieve stress (Burn and Shields, 2020).

In our study, in general, no statistically significant differences were found between groups because they were made up of the ideal number of animals to maximise welfare (less than 8 rabbits per cage) (Combes and Lebas, 2003), and there were therefore no significant differences in body temperature due to the effect of stress. Young rabbits are usually reared in groups (Lebas *et al.*, 1997), and a greater level of welfare of the rabbits is achieved when the group size does not exceed 6 animals (Morisse and Maurice, 1997).

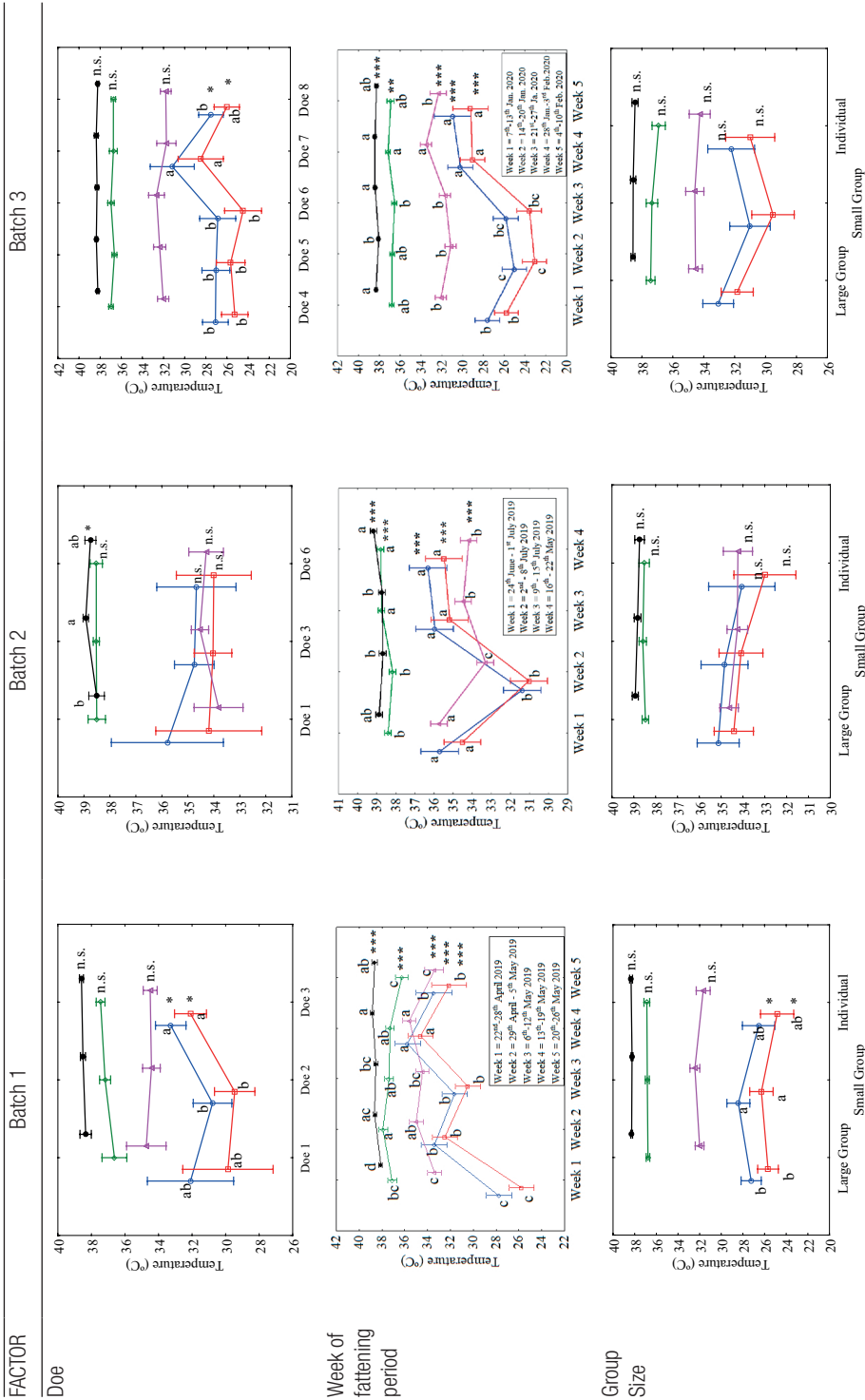


Figure 4: Thermographic (inner ear \blacksquare , outer ear \blacksquare , eye \odot and nose \blacktriangle) measurements, univariate General Linear Model, comparison within batch and according to doe, week of fattening period and group size (indicated with *P*-value). Duncan *post-hoc* analysis between means (indicated with letters).

a-c: Different letters indicate statistically significant differences between means within method ($P < 0.05$); n.s. = Not statistically significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Camacho *et al.* (2003) observed that the best weight gains are obtained when 7 rabbits are kept together in one cage in autumn-winter and 5 rabbits per cage in spring-summer. However, they found no differences in the average fattening weights in densities of below 8 rabbits per cage. On the other hand, when the group size exceeds 4-5 rabbits per cage, health risks increase and animal welfare is affected (Szendrő and Dalle Zotte, 2011). Furthermore, in large groups of rabbits, the haematological parameters are affected (lower erythrocytes, leukocytes and haemoglobin count, with higher platelet count), although the productive parameters are not, and the animals are therefore more sensitive to environmental effects. However, having groups of 8 animals from the same litter improves productivity and does not alter these parameters (Ramón-Moragues *et al.*, 2020).

Measuring body temperature by IRT requires specific equipment and software, which can be relatively expensive compared to conventional thermometers. Moreover, it involves the necessity to develop a learning curve to relate IR temperature with rectal temperature. In this context, the IRT technique reveals itself as a useful tool for fast and large-scale screening searching for early stages of diseases or stress in rabbit farms, where the high number of animals makes it expensive, in terms of the time required, to check the temperature of each rabbit using a conventional thermometer.

CONCLUSIONS

IRT is an effective tool for measuring the temperature of different regions of an animal's body, and these measurements are related to RT. The inner ear seems to be the best reference point for taking body temperature in fattening rabbits by IRT (IETT) due to higher correlations found with RT, while OETT measurement appears to be an inefficient way of representing body temperature, as it is used by rabbits to dissipate heat.

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Conflict of interest: The authors declare that they have no conflicts of interest.

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