SHORT COMMUNICATION

Using infrared ocular thermography as a tool to predict meat quality from lean cattle breeds prior to slaughter: Exploratory trial

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Abstract

Aim of study: To assess the potential of using infrared ocular thermography (IROT) as a tool to predict beef quality at the slaughterhouse.

Area of study: The study was carried out in the Salteras's slaughterhouse (Seville, Spain).

Material and methods: Ocular temperature images were captured from 175 lean young bulls prior to slaughter. Carcasses were classified into three groups according to weight: <250 kg, 250-310 kg and >310 kg. IROT was measured just before slaughter and pH was measured 24 h later. Colour parameters (CIELAB space) were evaluated 48 h post-slaughter. Water holding capacity was evaluated at seven days after slaughter.

Main results: IROT mean values were higher in heavier bulls ($p<0.05$), probably due to these animals appeared to movilize a greater blood flow, thus increasing ocular temperature. Furthermore, IROT showed a statistically significant correlation with both pH from light carcasses (r=0.66; $p<0.001$), and mean Hue value from all carcass weights (r=-0.22; $p<0.05$). A quadratic regression analysis accounting carcass weight as a continuous variable, found medium to strong fit values for pH (R^2=0.52; RMSE=0.032; $p<0.01$) and medium fit values for H* (R^2=0.41; RMSE=3.793; $p<0.001$), changing their relation with IROT depending on carcass weight.

Research highlights: IROT showed potential to become a useful tool to assess pH in light carcasses and to assess H* in all carcasses of young bulls prior to slaughter, regardless their weight. However, further studies would be recommended under more variable pre-slaughter stress conditions.

Additional keywords: eye temperature; Spanish native cattle breeds; beef quality; stress; water holding capacity; colour parameters, pH.

Abbreviations used: IROT (infrared ocular thermography); IRT (infrared thermography); LT (*Longissimus thoracis* muscle); RA (*Rectus abdominis* muscle); RMSE (root mean squared error); WHC (water holding capacity).

Competing interests: The authors have declared that no competing interests exist.

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Introduction

Infrared thermography (IRT) represents a non-invasive method used in numerous applications, not only in industry. Effectiveness of IRT has been proved for diagnostic purposes in animal diseases (Martins *et al.*, 2013), to assess physiological and metabolic parameters in production animals (McManus *et al.*,

2016) or to predict physiological variables related to the thermal stress of cattle (Viera de Sousa *et al.*, 2018).

When an animal becomes stressed, the hypothalamic–pituitary–adrenocortical axis is activated and heat is produced due to the blood flow increase, producing changes in heat radiation from the animal (Schaefer *et al.*, 2002). Ocular temperature is considered a good indicator of body core temperature (Johnson *et al.*,

2011) due to eyes are close to the brain. Thus, infrared ocular thermography (IROT) could be used to assess the neuro-physiological and stress conditions of animals at slaughter (Weschenfelder *et al.*, 2013; Losada-Spinosa *et al.*, 2018; Rocha *et al.*, 2019).

During pre-slaughter operations, livestock is exposed to a range of potential stressors, such as unfamiliar environment, human contact and transportation, which may compromise their welfare and impact meat quality traits. Furthermore, stressors factors at the slaughterhouse increase physical activity of the animals causing an increase in metabolic activity, heat production, increase of blood flow and depletion of muscle glycogen, leading to increase ultimate pH in meat (Eriksen *et al.*, 2013).

Beef quality is increasingly important as a large supply chain, and it raises concerns of a demanding fresh meat industry. Therefore, avoiding the appearance of bovine meat with high pH is important for the meat industry to decrease the appearance of quality defects (Loredo-Osti *et al.*, 2019). Thus, detecting stressed animals in the abattoir prior to slaughter would positively impact the meat industry.

The objective of this study was to determine whether the IROT estimates measured immediately before slaughter, under controlled welfare animal conditions, could be used to predict beef quality in light young bulls.

Material and methods

Animals

The study was carried out in a commercial slaughterhouse (Salteras, Seville province, Spain). A total of 175 yearling bulls (1-year-old and 450-550 kg live weight) from 9 farms located 80 km around the slaughterhouse, were randomly chosen for this study. In order to represent the southern Spanish market, all animals were Retinta × Limousin or Charolais crossbred. Young bulls were transported by road at night in compliance with animal welfare regulations (EC, 2005). The average duration of animal's transportation to the slaughterhouse was around 3 h including loading and unloading. After lairage (12 h) in individuals pens and non-skid floors (12 h, with water available but no food), young bulls were conducted at 8 a.m. to the point of stunning and were stunned using a captive bolt (Jarvis 25 Caliber, Standard, Captive Bolt, Pistol-Style Stunne, Koch Butchers Supply Company, USA). Then, they were dressed according to the guidelines of EC (2009).

In accordance to the commercial classification weight system, carcasses were grouped as 'light' (<250 kg; n=55); 'medium' (250-310 kg; n=75), and 'heavy' (>310 kg; n=45) (Lonja Mercamadrid, https://www.feriasymercados.net/index.php/lonja/index/12).

Collection of infrared thermography images

IROT images were obtained using a handheld infrared camera (Therma Cam i70 0, FLIR Systems AB, Danderyd, Sweden), operated by a trained technician. Images were collected in the stunning box, just prior to stunning. The left eye of young bulls was scanned from a 90° angle at a distance of 20 cm. At least three images per animal were collected, selecting the image that provided the optimal operating conditions for analysis. To avoid the confounding effect of environmental conditions on ocular temperature, environmental temperature and relative humidity were recorded with a digital thermohygrometer (Extech 44550, Extech Instruments Corporation, Nashua, NH, USA). IROT images were analyzed with the image analysis software Therma Cam Researcher Pro 2.8 SR-2 (FLIR Systems AB, FLIR Systems, Inc., Sweden) in order to obtain the maximum temperature (°C) within the area traced around the eye, including approximately 1 cm surrounding the outside of the eye lids. The maximum eye temperature, corrected by the environmental temperature and relative humidity and by an emissivity value of 0.98 (Stewart *et al.*, 2007), was used for the analyses.

Blood variables

Two blood samples (10 mL each) per animal were collected, using BD vacutainer® tubes containing potassium ethylene-diamine-tetra-acetic acid as anticoagulant for the analysis of glucose levels. Glucose (mg/dL blood) level was determined by an enzymatic capillary glucometer (OneTouch Ultra; LifeScan, Inc., Milpitas, CA) made *in situ* at the slaughterhouse, from plasma samples (Alcalde *et al.*, 2017).

Meat quality assessment

After 24 h at 4°C, pH was measured at the caudal segment of the right *longissimus thoracis* (LT) muscle using a pH-meter Crison 507 equipped with a penetrating electrode (Crison Instruments S.A., Barcelona, Spain) associated with a temperature compensation probe. To evaluate carcass colour, lightness (L*), redness (a*), and yellowness (b*) were then recorded in the CIELAB space (CIE, 1986) at the *rectus abdominis* (RA) muscle using a Minolta spectrophotometer (CM-700d; Minolta Sensing Inc., Singapore, Japan) with a 25-mm aperture, a specular component included, 10° view in angle, 0%

UV and D65 standard illuminant. Chroma (C*) and hue (H*) indexes were calculated as C*= $(a*^2+b*^2)^{0.5}$ and H*=$\tan^{-1}$ (b*/a*) and were expressed in degrees (MacDougall, 1986).

Seven days after slaughter, around 50 g of RA muscle was removed from the carcass and water holding capacity (WHC) was measured using the filter paper press method (Grau & Hamm, 1953). WHC was reported as % of juice expelled from fresh meat.

Statistical analyses

As physiological and meat quality parameters satisfied assumptions of normal distribution (results not shown), parametrical statistical analyses were used. In order to test the influence of the carcass weight level on the IROT measurements, a previous main effects ANOVA test was developed (model included weight as a fixed effect and no random effects), with this parameter showing statistically significant ($p<0.05$) differences for all the variables. According to that, differences due to the weight level of the animal within IROT measurements, blood glucose levels and meat quality parameters were assessed using a Duncan's post-hoc test. Pearson's correlations between IROT measurements and meat quality parameters were calculated for each weight level considered, in order to ascertain a relationship within them. Finally, in order to predict meat quality values from IROT measurements, a quadratic regression analysis was developed for those previous variables that resulted statistically significant ($p<0.05$) on the correlation analysis, accounting for the weight as a continuous variable. The quadratic equation with the coefficient of determination (R^2), and the root mean squared error (RMSE) and p value of the analysis were provided for each parameter. Statistical analyses were performed using the Statistica v.12.0 (Statistica software, v.12.0. Statsoft, Inc. 1984-2014) package.

Results and discussion

Means values of IROT and meat quality parameters according to three carcasses weight levels are shown in Table 1. Overall, IROT values obtained for the three carcass weight ranges were lower (33±4ºC) than reported by others authors (37.5±1.2ºC, Church *et al.*, 2014), probably due to lower environmental temperatures at the slaughterhouse (5-10ºC in this study *vs* 20ºC reported by Church *et al.*, 2014) and breed differences (Retinta x Limousin or Charolais crossbred *vs* Angus crossbred and Holstein cows).

A higher ($p<0.05$) ocular temperature was found in heavier carcasses compared with light and intermediate carcasses. These differences in IROT could be due to vessels' diameter, which is smaller in light young bulls, resulting in smaller blood flow in these animals compared to heavier young bulls. Behavioral reactions to stress suffered prior to slaughter induce a fast depletion of glycogen from the liver, increasing blood glucose levels and, indirectly, pH values (Lacourt & Tarrant, 1985). High pH and glucose levels in blood are associated with dark-cutting beef (Lu *et al.*, 2018).

In our study, glucose levels (53-194 mg/dL blood) and pH (5.4 to 5.9) were in the range of unstressed animals (Lu *et al.*, 2018). Morgan *et al.* (1995) reported that cattle whose IROT measures were outside the test temperature range (28-32 ± 2 °C) could be rejected as having a high probability of producing poor meat quality. In our study (Table 1), higher IROT ranges (29.2-37.3) would, *a priori*, predict a stress response that should affect meat quality. However, according to pH and glucose values, no stress signs were found in carcasses. This could be due to differences in animal responses to stress according to breed and weight factors that would condition the emission of temperature by the caruncle and hence, the temperature measured by the infrared camera. Amakiri (1976) reported a more superficial

Table 1. Descriptive statistics (means ± standard deviation and minimum-maximum intervals) and Duncan's post-hoc test within parameters, for infrared thermography camera measurements (IROT) and meat quality parameters according to different carcass weight groups of lean young bulls.

	IROT (ºC)	Meat quality parameters						
		pH[1]	L*[2]	a*[2]	b*[2]	C*[2]	H*[2]	WHC[2]
Min.-Max.	29.2-37.3	5.46-5.95	15.12-38.36	9.52-19.24	3.39-22.55	10.11-28.50	31.51-58.46	3.78-17.31
Carcass weight groups								
<250 kg	33.09±1.83[b]	5.55±0.01	32.76±2.56	15.51±1.79[a]	8.43±1.44[a]	17.66±2.23[a]	49.50±3.03[a]	8.69±2.52
250-310 kg	33.74±1.66[b]	5.57±0.08	32.74±2.41	14.66±1.48[b]	7.66±2.30[ab]	16.62±2.39[ab]	46.59±4.02[b]	8.72±2.94
>310 kg	35.03±1.09[a]	5.57±0.07	33.07±1.95	14.12±1.98[b]	7.03±1.59[b]	15.79±2.45[b]	45.41±4.50[b]	9.53±2.63
Total	33.91±1.72	5.56±0.07	32.83±2.33	14.72±1.74	7.68±2.01	16.66±2.44	46.97±4.18	8.92±2.77

L* = Lightness; a*= Redness; b*=Yellowness; C* = Chroma; H* = Hue; WHC = Water Holding Capacity (expressed as % of liquid expelled). [1]Measured on *longissimus thoracis* muscle. [2]Measured on *rectus abdominis* muscle. Different letters (a, b) within columns indicate statistically significant differences between means ($p <0.05$).

vascular arrangement in rustic cattle breeds, that could certainly bias the temperature captured by this tool due to the deeper vessels.

Regarding physical parameters of meat quality, colour values were within the ranges reported for commercial carcasses of young bulls from Southwest of Europe (Peña *et al.*, 2014). No significant differences ($p>0.05$) were found in the beef luminosity between the carcass weight groups. However, differences between means due to carcass weight were observed for a*, b*, C* and H* attributes due to a lower fat content expected in light carcasses compared with heavier carcasses (Gagaoua *et al.*, 2018).

Except for pH and H* colour values, no significant correlations were found between IROT and meat quality traits on each carcass weight group (Table 2). The IROT value was moderately and positively correlated with pH measured in the LT muscle of light carcasses (r=0.66; $p<0.05$). Thus, in case of light carcasses, a higher pH value in meat was expected in animals showing higher IROT values previous to slaughter. On the other hand, the correlation between IROT and H* value was weak and negative, although statistically significant (r=-0.22; $p<0.05$) in each weight group tested. Thus, regardless the slaughter weight, low H* values could be expected in beef from bulls showing high IROT values before slaughter. This observation can be explained due to the intraclass variability in each weight group tested was small, whereas the interclass variability observed was high.

Figure 1 shows the quadratic regression analyses for both parameters that showed statistically significant ($p<0.05$) correlations with IROT (pH and H*). For this analysis, weight was considered as a continuous variable. Results shown in Figure 1a indicate that carcass pH after slaughter could be predicted from IROT measurements obtained before slaughter, following the quadratic equation: pH = $7.231-0.0109*x-0.0507*y+2.8455E-5*x*x-3.2054E-5*x*y+0.0011*y*y$ ($R^2=0.52$; RMSE=0.032; $p<0.01$), where y is the weight of the animal and x

is the IROT measured before slaughter. As regards to Hue value, it could be predicted following the quadratic equation showed in Figure 1b: H* =$-25.4711+0.0831*x+4.2168*y+0.0003*x*x-0.0084*x*y-0.0334*y*y$ ($R^2=0.41$; RMSE= 3.793; $p<0.001$), where y is the weight of the animal and x is the IROT measured before slaughter.

Morgan *et al.* (1995) already found IROT as a useful tool for the early detection of poor meat quality in cattle before slaughter. They proposed to reject animals with IROT measurements differing at least 1.20 times from the mean IROT measurement calculated for the whole group slaughtered together. The quadratic regression analysis showed differences in the effectiveness of this tool due to animal weight. Furthermore, this analysis supported previous correlation results showing that the higher the IROT, the higher the pH value within the lightest animals' weight group (<250 kg), with a medium-strong relation ($R^2=0.52$). This could be due to the characteristics of the Spanish native breeds used for the analysis, in general lean and more heterogeneous in weight than those used by previous studies (mean body weight of 283±100kg in this study, *vs* 500±2kg reported by Morgan *et al.*, 1995, in Canadian bull breeds or 309±5kg reported by Church *et al.*, 2014, in Angus cross steers). Thus, the IROT interval previously proposed should be taken with caution in rustic animals. These results are supported by previous studies that found differences in meat quality and muscle structure of rustic compared with more selected cattle breeds (Vieira *et al.*, 2007).

Regarding meat colour, our results seem to suggest that low Hue values, related to redder carcass tone, could be linked to a greater blood flow through the muscles during slaughter, as a result of the HPA axis activation after a stress response, indicated indirectly by the high IROT values obtained (Stewart *et al.*, 2007). Despite, this relation was weak when just a linear correlation was considered (r=-0.22), a medium relation between H* and IROT value was found ($R^2=0.41$), when carcass weight was considered as a

Table 2. Pearson correlations between infrared thermography camera measurements and meat quality parameters for the three carcass weight levels in lean young bulls.

Carcass weight	pH[1]	L*[2]	a*[2]	b*[2]	C*[2]	H*[2]	WHC[2]
<250 kg	0.66***	0.17	-0.17	-0.09	-0.14	0.02	0.24
250-310 kg	-0.07	-0.08	0.12	0.01	0.07	-0.12	-0.09
>310 kg	-0.11	-0.26	-0.30	-0.33	-0.31	-0.28	-0.35
Total	0.07	-0.01	-0.15	-0.15	-0.15	-0.22*	0.01

L* = Lightness; a*= redness; b*= yellowness; C* = Chroma; H* = Hue; WHC = Water Holding Capacity; $p>0.05$; * $p<0.05$; *** $p<0.001$. [1]Measured on *longissimus thoracis* muscle. [2]Measured on *rectus abdominis* muscle.
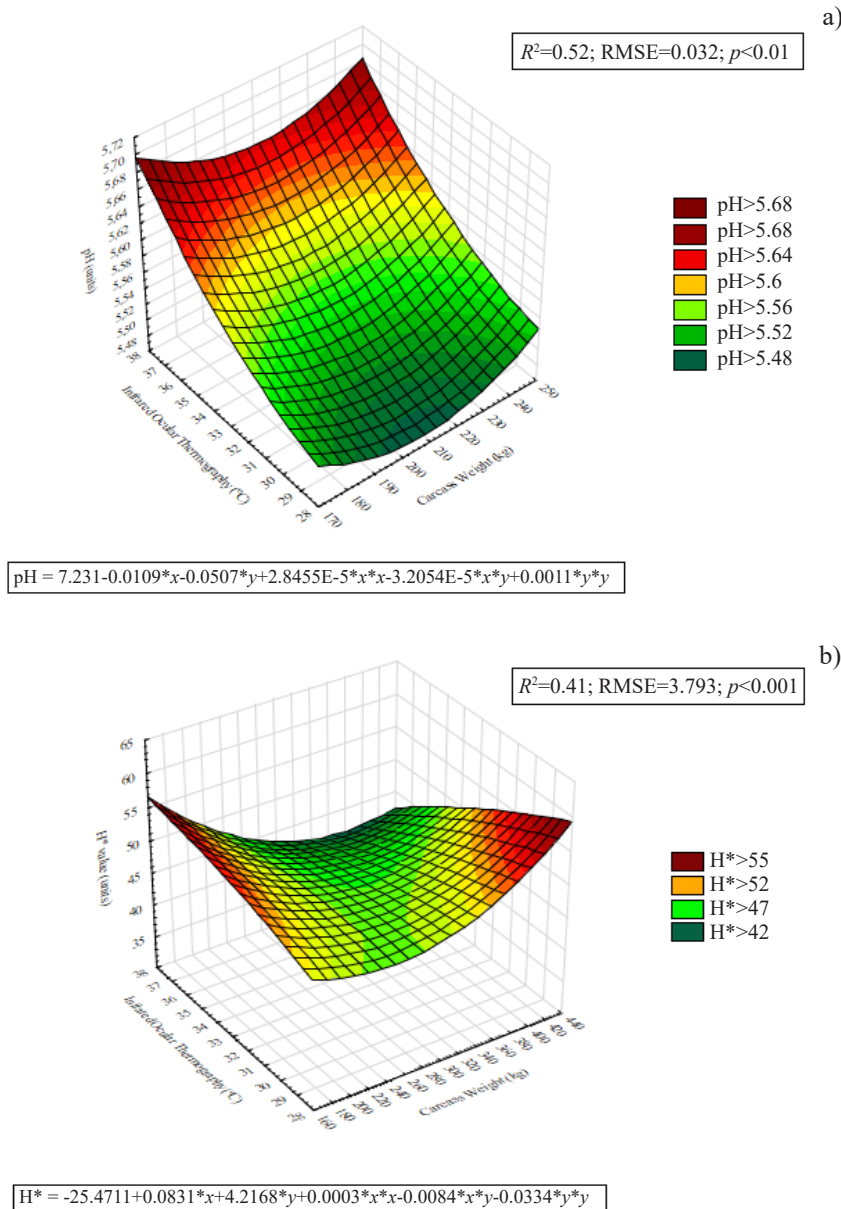
a)

$R^2=0.52$; RMSE=0.032; $p<0.01$

pH>5.68
pH>5.68
pH>5.64
pH>5.6
pH>5.56
pH>5.52
pH>5.48

$pH = 7.231-0.0109*x-0.0507*y+2.8455E-5*x*x-3.2054E-5*x*y+0.0011*y*y$

b)

$R^2=0.41$; RMSE=3.793; $p<0.001$

H*>55
H*>52
H*>47
H*>42

$H* = -25.4711+0.0831*x+4.2168*y+0.0003*x*x-0.0084*x*y-0.0334*y*y$

Figure 1. Quadratic regression analyses between infrared ocular temperature (x axis), carcass weight measurements (y axis) and meat quality parameters that resulted statistically significant on the previous correlation analysis (z axis): (a) for pH value; (b) for Hue (H*) value. The square in the bottom left side of each figure showed the quadratic equation that predicts pH and H* values, whereas the square in the upper right side of each figure showed the model fit parameters R-squared (R^2), root mean square error (RMSE) and p-value.

continuous variable, so that, the higher the weight, the higher the H* value and the lower the IROT temperature, thus supporting previous results obtained from the negative linear correlation.

The pre-slaughter use of IROT in bulls from lean breeds could be a potential low-cost and quick decision-making method to reduce meat quality defects due to pre-slaughter stress. Our preliminary results indicate a tendency of this instrument to change its effectiveness depending on carcass weight, showing better prediction potential with H* value regardless the weight and with pH on light lean young bulls. However, this technique will need further validation to confirm the correlations observed in the present study (including variability in pre-slaughter conditions and data from stressed animals) and to achieve the level of automation required to operate at line speed in a commercial slaughterhouse.

Acknowledgements

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