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Slaughtering of heifers in a local or an industrial abattoir: Animal welfare and meat quality consequences

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HIGHLIGHTS

- Slaughtering in small-scale local vs large-scale abattoir reduced animal stress.
- Further improvements of animal welfare are needed in both commercial systems.
- Consumers could differentiate steak colour from the different slaughter systems.
- Meat from local abattoir has improved palatability and shear force values.
- Final pH is not thought to be the main contributor to the meat quality differences.

GRAPHICAL ABSTRACT

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ABSTRACT

The aim of the study was to compare stress response and meat quality related to two abattoirs types. Pirenaica heifers were slaughtered in a small-scale local abattoir (LOC, n=8) or in a large-scale industrial abattoir (IND, n=8). The two groups differed in terms of transport distance and duration, waiting time, stunning to slaughter time, facilities, handling and processing conditions. Blood parameters showed higher levels of glucose, neutrophil/lymphocyte ratio, and neutrophils in animals slaughtered at the IND compared to the LOC, but cortisol levels were high in both groups. Meat colour from the LOC treatment was lighter, less red, yellow and saturated and had a greater hue angle than the IND, but colour deterioration showed a similar trend. LOC samples had lower shear force values and better eating quality compared to IND samples at day 11 of ageing. This study shows that slaughtering animals in small abattoirs, located close to farms, may reduce animal stress compared to large-scale industrial abattoirs, but there is room for improvement in both systems. Consumers may be able to differentiate steak colour from the different slaughter systems and find the meat from local abattoir animals more palatable. Final pH is not thought to be the main contributor to the differences.

1. Introduction

The world population is expected to reach nine billion by 2050, and thus an increase in protein demand and production is likely to occur (Tomlinson, 2011). If this demand is sustained over time, it is inevitable that production systems will become increasingly industrialised in the

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future in order to optimise the efficiency of processes, decrease production costs and improve food safety. From a conventional business point of view this situation has positive aspects, such as being able to provide a more homogeneous product and a constant, sustained offer over time. This trend leads to a standardisation of industrial processes, where generally large, specialised beef farms supply to few large-scale slaughterhouses (Sørensen et al., 2006). The question is whether these industrialisation processes affect animal welfare and the sustainability of the system. It is also important to know how this situation could affect the quality of the meat, by considering quality, in a broad sense, to include ethical aspects and welfare conditions in which the product is obtained (María, 2006). In several rural regions of Europe, there are still family farms and local producers that trade with nearby local abattoirs. These regional slaughterhouses (or sometimes mobile abattoirs) support local food production or short food supply chains, where calves are born, finished, slaughtered, processed and consumed nearby (Díez-Vial and Álvarez-Suescun, 2011). In this regard in recent years, public and private initiatives have been implemented where products are termed 'zero kilometre' or local foods (MAGRAMA, 2018), which appeals each day to more consumers (Akaichi et al., 2020). Among other differences, in these small-scale local production systems animals usually have more access to grazing areas and the distances to the slaughterhouse are short; in contrast, industrial slaughterhouses generally have better cold equipment and more optimised processes and safety management (Bustillo-Lecompte and Mehrvar, 2015). However, as there is only a small number of these very large slaughter plants, they tend to be located further away from the production centres.

One main challenge in beef cattle production is to know how pre- and post- slaughter handling affects animal welfare and the quality of the final product in each system. Several stress factors associated with pre-slaughter handling have been identified as being responsible for muscle glycogen depletion and high meat pH, such as animal handling, transport to slaughterhouse and lairage environment (Hambrecht et al., 2005; Bee et al., 2006; Romero et al., 2013; Coombes et al., 2014). This multi-factor stressor can increase the occurrence of dark cutting, associated with dark, firm and dry (DFD) syndrome in the meat obtained, and consequently affect microorganism growth (Koutsoumanis and Sofos, 2006), meat colour (Abril et al., 2001), tenderness (Silva et al., 1999) and consumer acceptability (Viljoen et al., 2002); which means that DFD steaks are not sold directly to consumers. All this can significantly affect farmers' economic income, and has a much more severe impact on smaller producers.

Despite a large number of studies reporting the effect of different factors on animal stress response and abnormal meat pH, there is a lack of information on the interaction between these pre-slaughter and processing factors, comparing small-scale local and large-scale industrial abattoirs, in relation to the final quality of the product perceived by consumers. Gebresenbet et al. (2011) report on the optimisation analysis conducted to determine the benefits of establishing a small-scale local abattoir in comparison to a large-scale abattoir in relation to transport distance and time of animal transport and meat distribution, which influence consumers' preferences and greenhouse gas emissions generated from vehicles. Eriksen et al. (2013) reported improved animal welfare and instrumental texture in lambs slaughtered in local (mobile) abattoirs *vs* conventional slaughterhouses. Additionally, a recent study showed that eating quality of meat from pH compliant carcasses (with pH_{24h} less than 5.8) could be influenced by different pre-slaughter management systems (Loudon et al., 2019).

It is expected that animals with a less stressful pre-slaughter environment and an adequate carcass handling and refrigeration will result in meat with a better sensory quality, as perceived by consumers. Therefore, the aim of this study was to evaluate the influence of two different slaughtering systems (small-scale local *vs* industrial) on stress response variables and meat quality, including consumer acceptability, in beef heifers. The working hypothesis is based on the idea that local and nearby production can have benefits both for animals and for farmers and consumers, bearing in mind that the role this type of production plays is supplementary to industrial production which for the time being and given the growing demand for meat, will continue to be necessary if there are no changes in the population's consumption habits.

2. Materials and methods

This study was conducted with animals reared under commercial farming practices following national regulations on animal welfare (Council Directive 2008/119/EC), with animals being slaughtered by following the European Union regulation 1099/2009 to protect animal welfare at the slaughterhouse. Ethical review and approval were waived for this study since practices were undertaken for the purposes of recognized animal husbandry, not likely to cause pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice. The care and use of animals were performed in accordance with the Spanish Policy for Animal Protection RD53/2013, which meets the European Union Directive 2010/63 on the protection of animals used for experimental and other scientific purposes.

2.1. Animals, experimental design and pre-slaughter conditions

A total of 16 Pirenaica purebred heifers, reared in the Pyrenean mountainous region of Sobrarbe (Huesca Province, North Spain), were used in the study. When born (calving session December-April), calves were reared with their grazing dams until weaning at 6 months. Thereafter, all animals were reared under conventional intensive conditions (feedlot), allotted with similar body weight (averaging 250 ± 30kg) in pens (15-25 animals) and fed concentrate-based diets (<12% wheat straw). After an adaptation diet of 2 weeks, animals were fed with a growth concentrate diet (14% crude protein, 38% starch and 4.5% fat) until 350 kg live weight, followed by a finishing concentrate diet higher in energy (13% crude protein, 66% starch and 5% fat) until the slaughter at 540 kg with an average age of 11.3 (± 1.53) months old.

The experimental design consisted of two different slaughter groups, one being slaughtered on an industrial abattoir and the other on a local abattoir. However, we also wanted to take into account pre–slaughter conditions related to each abattoir by following the conventional practices (ie. transport, waiting time, and handling) of these two slaughtering environments. Therefore, animals for the local slaughterhouse (LOC, n=8) were transported individually from the fattening unit to the slaughterhouse according to local market demand (1-2 per week). This occurred at the beginning of autumn with no rain and a range of daily temperatures between 15 and 25 °C. Transport was carried out in small trucks (3-3.5 m long x 1.5-2.5 m wide) always with the same driver. The local slaughterhouse (LOC) was less than 10 km from the fattening unit (Matadero Municipal de Aínsa), and animals were slaughtered immediately upon arrival. In contrast, animals for the IND (industrial slaughterhouse, n=8) group were transported from the fattening unit to the slaughterhouse all together the same day in a commercial truck 9.5 m long x 2.5 m wide. In addition, the large-scale industrial slaughterhouse (MercaZaragoza) was located at a distance of 220 km from the fattening unit and animals had to wait before slaughtering because of the usual working volume. Animals from other origins were not mixed in the waiting pen and had water to drink. These animals were slaughtered in the middle of autumn with no rain and similar temperature range as LOC animals. Transport time, from loading animals in the truck until unloading at each abattoir, and waiting time were recorded with a manual chronometer.

2.2. Slaughter and post-slaughter conditions

The two abattoirs differed in animal handling, processing conditions and industrial equipment. The LOC abattoir counted for two operators

who stunned, bled and manually processed the carcass of animals. This process was carried out in a small cutting room without climate regulation and it took approximately an hour per animal. In contrast, the large-scale abattoir (IND) counted for on average 25 operators in the slaughter chain, being capable of stun, bled and process 25-30 carcasses per hour in a cutting plant with controlled temperature (7°). Maximum and minimum temperature was measured in the cutting/dressing room of each abattoir during carcass dressing by a hand thermometer. The stunning method was the same in both groups: penetrating captive bolt (Blitz-Kerner) 22 caliber in a slaughter box. The time from stunning to bleeding was recorded using a hand chronometer, from the sound of the bullet until the animal was lying for bleeding. Carcass hanging was through the Achilles tendon in both slaughterhouses and no electrical stimulation was applied. Carcasses of animals were stored with fixed temperature (4°) in cold chambers at both slaughterhouses.

2.3. Blood, serum and pH determination

At each abattoir, two blood samples per animal were taken in two different tubes to evaluate physiological stress (10 ml tubes, with and without anticoagulant, EDTA-K3). Samples were kept on ice for a maximum of 2 h and taken to the laboratory for routine haematological measurements. EDTA plasma and serum were centrifuged at 3000 rpm for 10 min and aliquots were frozen and kept at −30°C until analysis. An automatic particle counter (Vet-ABC, Divasa Farmavid S. A.) was used to count red blood cells (RBC) and white blood cells (WBC) (number per mm^3), haemoglobin (g/dL) and haematocrit (%). The leukocyte formula was estimated from blood swabs on clean slides. Staining was performed by the rapid panoptic method using dyes from Química Clínica Aplicada Inc. (QCA). With an optical immersion microscope, 100 leucocytes per sample (neutrophils, lymphocytes, eosinophils, basophils and monocytes) were identified and counted. The neutrophil/lymphocyte ratio (N/L) was used as a chronic stress indicator (Drazner, 1987; Lawrence and Rushen, 1993). Concentration of glucose (mg/dL, Ref. Glucose AE2-17) was determined with serum samples, as was the activity of creatinine kinase (CK), (UIL−1) (Ref. CK.NAC AE1-13) using an ACE® (Alfa Wasserman Clinical Chemistry System) multi-analyser and reagents. Cortisol concentration was determined from plasma (EDTA-K3) by enzyme immunoassay following (Miranda-de la Lama et al., 2010) using an 'in home-kit' (validated by Chacón et al., 2004). Each sample was determined in duplicate from 50 μL of plasma and the results were expressed in mol/L, with the corresponding controls. Inter- and intra-assay coefficients of variation of the analysis were 7 and 8 % respectively. The concentration of lactate was determined using a Sigma Diagnostic kit (lactate no. 735-10) and spectrophotometer (Lambda 5, Perkin Elmer). Serum concentration of non-esterified fatty acid (NEFA) levels was analysed by an ACE® (Alfa Wasserman Clinical Chemistry System) multi-analyser with commercial kits (NEFA C Ref. 994-75409 of the Wako).

To determine the pH of the *Longissimus* muscle, we used a portable pH meter (fitted with a penetration electrode 52-00, Crison), which was inserted into a small incision in the right loin muscle between the 7th and 9th ribs at 24h post-slaughter. The pH meter was re-calibrated after every five samples, using two standard buffer solutions at pH 7.02 and 4.00.

2.4. Carcass characteristics and meat sampling

Cold carcass weight was obtained 24 hours after slaughter and conformation and fattening score were evaluated following European meat standards ((EU) 2017/1182). The left *semitendinosus* muscle from each animal was excised and transported to the Meat Laboratory at the Faculty of Veterinary of the University of Zaragoza, where it was vacuum packed and stored at 4 °C until reaching 4 days of ageing. Following assessment of pH the muscle was sampled for chemical composition, colour, texture and consumer analyses.

2.5. Chemical composition

Vacuum-packed samples of the *semitendinosus* muscle (40 g) were frozen and transported to an external laboratory for proximate analysis (INTA, Teruel, Zaragoza), where they were kept frozen until analysis. After thawing by placing the samples at room temperature for 24 h, maintaining vacuum conditions, samples were minced and homogenised prior to determination of dry matter (ISO 1442:1997), total fat (ISO 1443:1973), protein (ISO 937:1978) with a conversion factor of 6.25 and ashes (ISO 936:1998). All analyses per animal were performed in duplicate.

2.6. Meat colour

A 3 cm-thick steak from each animal was used for colour measurements. Analyses were conducted with a Minolta CM 2002 spectrophotometer (Konica Minolta Holdings Inc., Osaka, Japan), in the CIE L*a*b* space (CIE, 1976) with 10° viewing angle and D65 illuminant. Individual steaks were placed in plastic foam trays and wrapped with oxygen-permeable film without touching the surface of the muscle. Three measurements were taken in different locations of the cutting surface and the average was recorded after 1 h of blooming and following 1, 2, 3, 4, 7 and 10 days of storage in a refrigerator at 4°C and in dark conditions. Chroma $= \sqrt{a^{*2} + b^{*2}}$ and hue angle $=$ arctg $(\frac{b*}{a*})$ in degrees were calculated. Reflectance between 360 nm and 740 nm were recorded at 10-nm intervals, selecting two to calculate R630 minus R580 as an indicator of discoloration.

2.7. Texture analyses

Four 3 cm-thick steaks from the *semitendinosus* muscle from each animal were obtained and vacuum packed individually. Half of them were kept at 4 ºC until 4 days of ageing, and half of them until 11 days. All samples were stored at -18 °C until analysis. After thawing for 24 h at 4°C the texture was analysed using an INSTRON 4301 (Instron Limited Corporation, High Wycombe, United Kingdom) with a modified compression cell using raw meat and a Warner–Bratzler fixture for cooked meat (Campo et al., 2000). The meat was cut into rectangular pieces of 1 cm^2 cross-section, perpendicular to the direction of muscle fibres. With the compression device, the stress was assessed at 20% (C20) and 80% (C80) of maximum compression; whereas maximum load (shear force) and toughness (amount of force necessary to break the samples) were measured using the Warner-Bratzler device. Freezing and cooking losses were calculated in the same steak samples of the Warner-Bratzler test, obtained by weighing the meat before frozen and after thawing, and before and after being heated in a water bath at 70°C respectively.

2.8. Consumer analysis

The test involved a total of 80 consumers selected based on the Spanish demographic characteristics (INE, 2016) regarding gender (55% males, 45% females) and age (25.0% of the individuals were <30 years old, 31.3% were between 31 and 44 years old, 21.3% were between 45 and 59 years old and 22.4% were >60 years old). The day before each session, the frozen samples (2 cm-thick *semitendinosus* muscle) previously aged for 4 and 11 days were thawed for 24 h at 4°C. To achieve an internal muscle temperature of 13-15°C, steaks were kept at ambient temperature (20°C) for ~1 h before they were cooked on a pre-heated, double-grill hotplate at 200°C (SAMMIC GRD10, SAMMIC S. L., Guipuzcoa, Spain) until the internal temperature reached 70°C, which was monitored using a JENWAY 2000 (Bibby Scientific Ltd, Stone, UK) internal thermocouple. Subsequently, 10 homogeneous cubes per steak were obtained, wrapped individually in aluminium foil, marked with a unique three-digit code and kept warm at 50°C for less

than 10 min until they were served. Each consumer evaluated four samples from LOC and IND groups, each group with 4 and 11 days of ageing, and tasted them in random order to avoid the effect of sample order presentation, first-order or carry-over effects (Macfie et al., 1989). Consumers were asked to eat salt-free toasted bread and to rinse their mouths out with water before tasting each meat sample. Consumers were given instructions before the test and were supervised to ensure that the proper procedures were followed. The variables assessed were global, tenderness and flavour acceptability. The sensory intensity scale used a 9-point structured hedonic scale (dislike extremely to = like extremely), without the neutral central point (neither like nor dislike) obliging the consumer to make either a positive or a negative decision (Furnols and Guerrero, 2014).

2.9. Statistical analyses

First, all variables were analysed for normality using the Shapiro-Wilk test. The analyses of animal and carcass characteristics, *ante-mortem* variables, blood parameters, chemical composition and pH were analysed with a General Lineal Model (GLM), considering abattoir (LOC or IND) as fixed factor, using the SPSS (v22.0) software. For colour parameters a GLM model was applied, where abattoir, display time (1h, 24h, 48h, 72h, 4 days, 7 days and 10 days) and their interactions were considered as fixed factors. To analyse differences in meat colour by abattoir type, in each display time, the database was segmented and independent GLMs were conducted. Inversely, the effect of display time within abattoir was also analysed and, when significant, a Tuckey test was used to assess mean comparisons of display days ($P \leqslant 0.05$).

Texture, freezing and cooking losses were analysed with a GLM, considering abattoir and ageing for 4 or 11 days, and their interactions as fixed factors. For the consumer test, a mixed-effect model was applied with abattoir and ageing as fixed effects and consumer as a random effect. Consumer data did not follow a normal distribution ($P \leqslant 0.05$) and it was analysed by using a Kruskal-Wallis test on R, with days of ageing and slaughter type as fixed effects and consumer as random effect. Then, due to significant interactions, data was also analysed again with the interaction (ageing time x slaughter time) as fixed and consumer as random effects, with a posthoc (Kruskal Nemenyi test) to detect mean differences.

Finally, a Principal Component Analysis was carried out using the main variables (Standardized) related to animal stress and carcass and meat quality, also including all individuals (N=16), performed with R studio (R Core Team, version 1.1.463, 2018). The figure combined results from the individuals (re-scaled to a range of −1 to 1) with the variables in a biplot, completed in Microsoft Excel 2010.

3. Results

3.1. Pre/post-slaughter conditions of animals slaughtered in local vs industrial abattoirs

Pre- and post- slaughter conditions in the two groups studied are presented in Table 1. As the traveling distance from the fattening unit to the slaughter was inferior for LOC animals than IND animals (<10 km *vs* 220 km), the transport time was considerably minor. In addition, as IND animals were transported all together in the same travel we cannot achieve variability (SD) on transport, waiting time or fasting period. When animals arrived at the slaughterhouse, LOC animals waited less (-86%; P<0.001) time to be slaughtered. Once at the slaughterhouse, the time from stunning to bleeding was shorter (-21%; P=0.003) for LOC *vs* IND animals. With regard to the temperature measured in the dressing room while processing carcasses, minimum and maximum temperatures were around 10°C higher in LOC *vs* IND abattoir, where temperature was fixed. Furthermore, carcasses at the LOC remained in the dressing room longer (45 to 65 minutes *vs* 15 minutes at the IND), before entering the cold chamber where the temperature was set at 4°C.

Table 1
Mean and standard deviation for pre- and post-*mortem* variables of Pirenaica heifers slaughtered at a local (LOC) or industrial (IND) abattoir.

Variables	LOC	IND	*P* Value
Transport distance (km)	4.6 ± 1.9	220.0	<0.001
Transport time (m)	11.5 ± 5.3	180.0	<0.001
Waiting time (m)	8.5 ± 1.7	60.0	<0.001
Stunning to bleeding time (s)	36.9 ± 7.0	46.3 ± 2.3	0.003
Minimum temperature (°C)[†]	13.2 ± 0.7	3.0	<0.001
Maximum temperature (°C)[†]	17.2 ± 1.2	7.0	<0.001

[†] Minimum and maximum temperature of the dressing room during carcass dressing.

3.2. Blood parameters and stress variables of animals slaughtered in local vs industrial abattoirs

Stress response variables and *longissimus* pH values at 1 and 24 hours *post mortem* are shown in Table 2. Regarding red cells, LOC animals presented less (P≤0.02) haematocrit (-22%) and lower medium corpuscular volume (-7%) than IND animals. White cells showed that LOC animals presented fewer amounts of neutrophils (-58%; P<0.001) than IND animals, without significant changes in the other white cellules (P>0.13). Consequently, LOC animals showed a lower neutrophil/lymphocyte ratio (-66%; P=0.004) than IND animals. In addition, a higher platelet count was observed as a trend (P=0.10) in IND animals. Regarding plasma variables, only glucose presented higher (+34%; P=0.003) values in IND vs LOC animals. Finally, pH values significantly differed between treatments, *Longissimus* muscle pH presented higher (+2.5%; P=0.01) values one hour post-slaughter for LOC animals, but lower (-2.3%; P<0.001) at 24 hours post-slaughter in LOC compared to IND carcasses.

3.3. Carcass characteristics and chemical composition, colour and texture of meat of animals slaughtered in local vs industrial abattoirs

Carcass characteristics for the animals slaughtered and meat composition of the *semitendinosus* muscle from LOC or IND abattoirs are described in Table 3. Carcass weight and conformation were similar between treatments, but fatness was higher in the IND group. Non-significant differences (P>0.21) between treatments were observed in the chemical composition of the *semitendinosus* muscle in both groups.

Meat colour variables of *semitendinosus* steaks: L*, a*, b*, C* and

Table 2
Mean and standard deviation for blood parameters at exsanguination and pH measured in *longisimuss* muscle at 1 h and 24h post-slaughter of Pirenaica heifers from local (LOC) or industrial (IND) abattoirs.

	LOC	IND	*P* Value
Haematocrit (%)	40.3 ± 3.31	45.3 ± 3.91	0.01
Red blood cells (10^6/mm^3)	10.2 ± 0.91	10.3 ± 1.11	0.83
Haemoglobin (g/dL)	14.1 ± 1.43	14.9 ± 1.63	0.30
Medium corpuscular volume (fL)	40.9 ± 1.02	43.9 ± 3.02	0.02
White blood cells (/mm^3)	8107.7 ± 1489.7	9718.7 ± 2245.2	0.11
Neutrophils (/mm^3)	1738.8 ± 803.1	4143.7 ± 929.2	<0.001
Eosinophils (/mm^3)	130.3 ± 68.5	129.2 ± 73.4	0.97
Basophils (/mm^3)	83.94± 42.2	57.2 ± 21.1	0.13
Lymphocytes (/mm^3)	5632.0 ± 1489.7	4911.8 ± 1643.1	0.37
Monocytes (/mm^3)	518.2 ± 116.5	476.8 ± 213.1	0.63
Platelets (10^3/mm^3)	190.4 ± 93.5	288.9 ± 130.8	0.10
Neutrophils / Lymphocytes	0.33 ± 0.21	0.96 ± 0.47	0.004
Creatinine kinase (UI/L)	1139.1 ± 1441.8	646.0 ± 141.1	0.35
Glucose (mg/dL)	117.9 ± 17.2	178.4 ± 43.6	0.003
Lactate (mmol/L)	4.50 ± 1.41	3.88 ± 3.98	0.68
Cortisol (nmol/L)	178.9 ± 21.8	155.1 ± 41.0	0.16
Non-esterified fatty acids (mmol/L)	0.31 ± 0.12	0.35 ± 0.21	0.63
pH_{1h} *longissimus muscle*	6.95 ± 0.09	6.78 ± 0.12	0.01
pH_{24h} *longissimus muscle*	5.58 ± 0.55	5.71 ± 0.60	<0.001

Table 3
Mean and standard deviation for age at slaughter, carcass characteristics and meat pH and proximate composition of *semitendinosus* muscle from Pirenaica heifers slaughtered at a local (LOC) or industrial (IND) abattoir.

	LOC	IND	*P* Value
Age at slaughter (m)	10.7 ± 1.28	11.7 ± 1.66	0.20
Carcass characteristics			
Cold carcass weight (kg)	238.6 ± 22.4	225.6 ± 27.1	0.31
Conformation†	8.50 ± 0.92	8.38 ± 0.51	0.74
Fatness‡	5.50 ± 0.53	8.00 ± 0.00	<0.001
pH_{4d} *semitendinosus* muscle	5.55 ± 0.05	5.58 ± 0.06	0.30
Proximate composition			
Water (g/100g)	71.3 ± 2.41	73.3 ± 2.09	0.35
Crude protein (g/100g)	23.2 ± 0.89	23.3 ± 1.70	0.84
Crude fat (g/100g)	3.35 ± 1.22	2.65 ± 0.84	0.21
Ash (g/100g)	1.18 ± 0.06	1.16 ± 0.07	0.48

† SEUROP classification grid for carcass conformation scores from P- = 1 to S+ = 18
‡ SEUROP classification grid for carcass fatness scores from 1- = 1 to 5+ = 15.

Hue, and the index R630-R580, recorded at 1h and 1 to 10 days of display periods are shown in Table 4. Overall, LOC samples were lighter ($P<0.001$), less red ($P<0.001$), less yellow ($P<0.001$) and less saturated ($P<0.001$) than IND meat. In contrast, LOC meat presented greater ($P<0.001$) hue angle compared to IND, but no statistical effect was found for R630-580.

Texture characteristics measured with two instrumental devices and freezing and cooking loss of *semitendinosus* steaks aged in vacuum conditions for 4 and 11 days from LOC and IND abattoirs are shown in Table 5. Warner-Bratzler shear force (WBSF) in the cooked meat from LOC was lower ($P=0.009$) both at 4 days of ageing (-24%) and at 11 days of ageing (-13%) than IND meat. Fibres compression did not show differences between treatments ($P\geq0.74$), with however, a higher (33%: $P=0.006$) average C20% compression values at day 4 vs day 7. Finally, water holding capacity (WHC), measured as freezing and cooking losses, was not statistically affected by the slaughterhouse nor by the ageing times compared.

3.4. Consumer's results of meat of animals slaughtered in local vs industrial abattoirs

Consumer data is shown in Table 6. On average, consumer scores showed how on average, LOC meat presented at day 11 of ageing better ($P<0.05$) global (+15%), tenderness (+20%) and flavour (+10%) acceptability than IND meat. In addition, ageing up to 11 days influenced these consumer variables by increasing the three acceptability values evaluated only in the case of LOC but not in IND meat.

3.5. Relationships between pre/post-slaughter conditions, stress and meat characteristics

Figure 1 shows an overview of the main results obtained in this study in a biplot PCA with two components, explaining 46.3% of total variability. The PCA obtained discriminates LOC samples from IND samples; where LOC meat were associated with increased pH_{1h}, with lighter meat (at 24 h of display) and with greater consumer acceptability scores. All these variables have proved to be statistically affected by abattoir type (Tables 2, 4 and 6). Furthermore, the LOC samples were found to be more similar within the group compared to the IND samples, which showed greater dispersion. The IND meat presented higher variability among samples and was related to medium corpuscular volume (MCV), neutrophil/lymphocyte ratio (N/L), glucose level at exsanguination, higher *longissimus* muscle pH at 24 h post-slaughter, higher carcass fatness, more red (a*) and yellow (b*) meat at 24 h of display and tougher cooked meat, measured by Warner Bratzler shear force (WBSF).

4. Discussion

There is no global consensus to define local food in terms of specific distance from production to consumption, since consumers have their own understanding of the concept, which could vary between and within countries in a certain product (Zhang et al., 2020). Akaichi et al. (2020) defined local beef as that produced and consumed in the same autonomous region, which perhaps is a valid geographical boundary for some consumers, such as those living in big cities, but local beef could also be defined in a smaller area, considering the role that livestock can play in fixing the local population to the territory. Local beef in small areas means that animals are born, reared, finished, slaughtered and further processed regionally by using a small abattoir facility which is in charge of satisfying their beef demand. This small abattoir would avoid having to transport animals outside the region to an industrial slaughterhouse and to transport their products back again, for consumption at the original location. In this study we compare animal welfare and meat quality from animals slaughtered at a small-scale/local (LOC) abattoir *versus* a large-scale industrial (IND) abattoir.

Table 4
Mean and standard deviation for colour parameters of *semitendinosus* steaks under overwrap packing according to display time, from Pirenaica heifers slaughtered at a local (LOC) or industrial (IND) abattoir.

	L*		a*		b*		C*		Hue		R630-580	
	LOC	IND	LOC	IND	LOC	IND	LOC	IND	LOC	IND	LOC	IND
1 h	41.1^{a} ± 1.97	37.2^{b} ± 2.70	12.7^{bX} ± 2.38	16.4^{aX} ± 2.69	17.8^{X} ± 1.51	19.6^{X} 2.93	21.9^{bX} ± 2.25	25.6^{aX} ± 3.87	54.7^{aXY} ± 4.60	50.1^{bX} ± 2.15	19.5^{X} ± 1.85	19.3^{X} ± 1.90
24 h	42.5^{a} ± 2.02	40.2^{b} ± 1.73	14.0^{bX} ± 1.54	17.6^{aX} ± 2.65	17.7^{X} ± 2.08	19.4^{X} ± 2.74	22.6^{bX} ± 2.37	26.2^{aX} ± 3.50	51.7^{aX} ± 2.81	47.8^{bX} ± 3.34	19.5^{X} ± 1.96	20.5^{X} ± 1.86
2 d	43.2^{a} ± 2.02	40.3^{b} ± 1.98	12.8^{bX} ± 1.84	17.1^{aX} ± 2.12	17.6^{bX} ± 1.72	19.4^{aX} ± 1.95	21.8^{bX} ± 2.17	25.8^{aX} ± 2.77	54.1^{aXY} ± 3.48	48.6^{bX} ± 1.80	18.4^{bX} ± 1.18	20.4^{aX} ± 1.98
3 d	43.4^{a} ± 2.29	41.6^{b} ± 1.69	12.1^{bX} ± 1.40	16.0^{aX} ± 2.15	16.9^{bXY} ± 1.03	18.9^{aXY} ± 1.52	20.8^{bX} ± 1.11	24.8^{aX} ± 2.40	54.5^{aXY} ± 3.72	49.8^{bX} ± 2.52	18.7^{X} ± 3.77	20.4^{X} ± 2.09
4 d	43.1 ± 1.52	41.8 ± 2.02	11.5^{bXY} ± 1.41	14.9^{aXY} ± 1.77	16.3^{bXY} ± 1.40	17.7^{aXYZ} ± 1.83	20.0^{bXY} ± 1.70	23.1^{aXY} ± 2.40	54.7^{aXY} ± 2.97	49.9^{bX} ± 2.12	16.7^{XY} ± 1.92	18.3^{XY} ± 1.76
7 d	43.0 ± 3.67	42.6 ± 1.89	9.36^{bY} ± 0.77	11.8^{Y} ± 1.94	15.0^{Y} ± 1.51	15.8^{Z} ± 0.56	17.7^{bYZ} ± 1.34	19.7^{aYZ} ± 1.35	57.8^{aY} ± 3.43	53.5^{bX} ± 4.47	13.8^{Y} ± 2.63	14.7^{Y} ± 2.30
10 d	44.2 ± 3.22	42.4 ± 1.60	6.57^{Z} ± 1.24	7.68^{Z} ± 2.76	14.7^{bY} ± 1.02	16.0^{aYZ} ± 1.17	16.1^{bZ} ± 1.21	17.9^{aZ} ± 1.70	66.0^{Z} ± 3.74	64.7^{Y} ± 7.93	8.30^{Z} ± 2.82	8.09^{Z} ± 4.62
P Abattoir	<0.001		<0.001		<0.001		<0.001		<0.001		0.041	
P Display	0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
P A x D	0.40		0.30		0.96		0.66		0.81		0.78	

$^{a-b}$: different superscripts within a column denote statistical differences ($P\leq0.05$) between abattoir groups within display time for a colour parameter.
$^{X-Z}$: different superscripts in the same row denote statistical differences ($P\leq0.05$) between display time within abattoir group for a colour parameter.

Table 5
Mean and standard deviation for instrumental texture, freezing and cooking loss of *semitendinosus* steaks aged for 4 or 11 days, from Pirenaica heifers slaughtered at a local (LOC) or industrial (IND) abattoir.

	Day 4		Day 11		*P* Abattoir	*P* Ageing	*P* A x A
	LOC	IND	LOC	IND			
Compression							
C20% (N/cm^2)	9.89 ± 3.64^{Y}	10.30 ± 4.53^{Y}	6.56 ± 2.34^{Z}	6.89 ± 1.50^{Z}	0.74	0.006	0.97
C80% (N/cm^2)	60.1 ± 8.55	69.1 ± 13.91	60.4 ± 6.85	68.8 ± 20.13	0.79	0.99	0.96
Warner-Bratzler							
Shear force (kg/cm^2)	4.00 ± 0.89^{b}	4.97 ± 0.82^{a}	3.90 ± 0.59^{b}	4.41 ± 0.63^{a}	0.009	0.21	0.75
Water losses							
Freezing losses (%)	10.5 ± 2.32	8.87 ± 2.24	9.21 ± 1.65	10.2 ± 0.56	0.67	0.92	0.05
Cooking losses (%)	32.2 ± 4.14	30.1 ± 5.98	38.3 ± 10.99	32.7 ± 5.71	0.14	0.09	0.47

$^{a-b}$: different lowercase superscripts in the same row denote statistical differences ($P \leq 0.05$) between abattoir groups within ageing day.
$^{Y-Z}$: different uppercase superscripts within a row denote statistical differences ($P \leq 0.05$) between ageing days within abattoir group

Table 6
Mean and standard deviation for consumer data of *semitendinosus* steaks aged for 4 or 11 days, from Pirenaica heifers slaughtered at a local (LOC) or industrial (IND) abattoir.

Acceptability	Day 4		Day 11		*P* Abattoir	*P* Ageing	*P* A x A
	LOC	IND	LOC	IND			
Global	5.55 ± 1.37^{b}	5.38 ± 1.38^{b}	6.35 ± 1.04^{a}	5.42 ± 1.31^{b}	<0.001	0.010	0.030
Tenderness	5.70 ± 1.41^{b}	5.07 ± 1.55^{bc}	6.50 ± 0.13^{a}	5.22 ± 1.44^{c}	<0.001	<0.001	0.010
Flavour	5.81 ± 1.26^{ab}	5.56 ± 1.36^{b}	6.25 ± 1.22^{a}	5.67 ± 1.23^{b}	<0.001	0.005	0.010

^{a,b,c}: different superscripts in the same row denote statistical differences ($P \leq 0.05$).

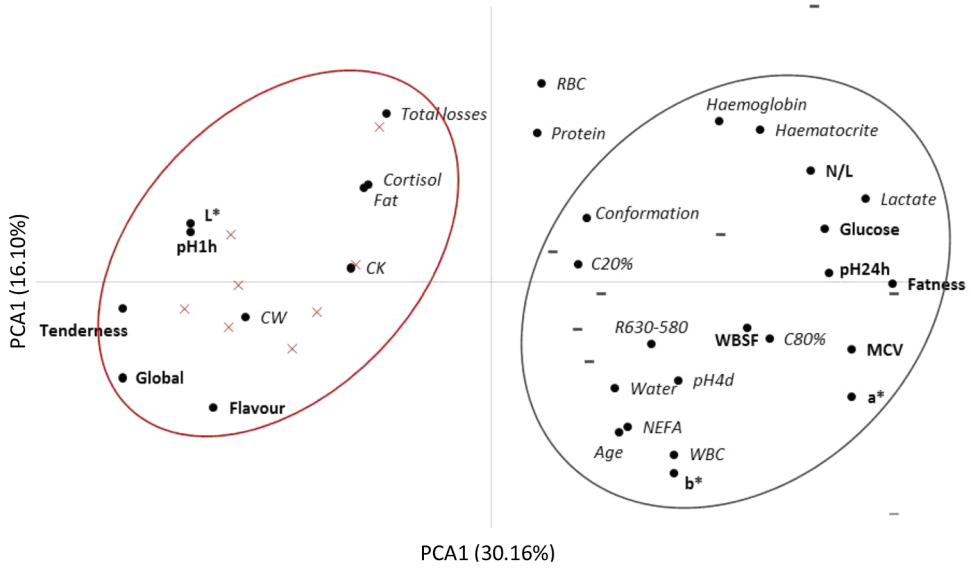

Figure 1. Principal Component Analysis of the main stress indicators, and carcass and meat quality data obtained from yearling Pirenaica heifers slaughtered at a local (LOC, **x**) or industrial (IND, **-**) abattoir.

4.1. Pre- and post-slaughter conditions of animals slaughtered in local vs industrial abattoirs

Pre-slaughter conditions which influence the most animal welfare and stress are mainly the fasting period, the transport time and waiting time in the slaughterhouse (Njisane and Muchenje, 2017). The fasting period, which is common practice consisting in feed withdrawal before slaughter, may cause hunger and metabolic stress, but since it lasts less than 12 hours in both groups, no relevant negative impact on physiological and haematological measurements is thought to have occurred (Earley et al., 2006). Distance travelled and time spent in transit from farm to abattoir, including loading and unloading of truck, were higher in the IND group, totalling three hours. However, transports averaging no more than three hours, as regular cattle transports in Spain, may not influence severe stress if carried out in proper conditions, as shown by previous studies (Villarroel et al., 2001; Mach et al., 2008). Authors determined that muscle glucogenic reserves were not affected in transport times before than 3 hours. The period from unloading animals at slaughterhouse until stunning (waiting time) has been considered either a stressful situation or a period necessary for the animals' physiological recovery from the previous acute stress undergone during transport, depending on various factors, such as transport time and conditions, management, equipment and microclimate conditions at the slaughterhouse (Miranda de la Lama, 2013). A previous study evaluating more than five thousand animals at an industrial slaughterhouse in Spain showed that the average waiting time was 12 hours and in those cases in which it was more than 15.8 hours, a significant increase in the frequency of animals with higher ultimate pH was observed, compared to those with lower waiting times (Mach et al., 2008). In our study, waiting time was different between the groups, when LOC animals arrived to the

abattoir they were almost immediately slaughtered while IND animals waited for 1 hour.

Regarding the main slaughtering factors affecting animal welfare or stress, the way of stunning the animals, which encompasses the number of shoots; the time between the stunning and the bleeding; and the animal handling on the abattoir are highlighted (Grandin, 2000). In both abattoirs, all animals were correctly stunned at the first shoot. However, differences were observed in the stunning to bleeding time because of the differences in animal management between both abattoirs. After stunning, at the industrial abattoir animals were hung on a rail and bled, while at the local abattoir bleeding was performed on the floor right after the animal falls (horizontal bleeding), and therefore the LOC achieved a shorter period from stunning to slaughter. Higher stunning to bleeding time may allow the animals to regain consciousness and has been associated with higher heart rate activity; and both these consequences could involve metabolic changes influencing animal welfare and stress (Vimini et al., 1983; Grandin, 2000). Indeed, some animals from the IND group showed leg and head movements after stunning, although none of them presented signs of consciousness, and in both treatments the interval between stunning and exsanguination was no more than 60 seconds, which is the maximum time recommended by the humane slaughter association (HSA, 2013).

Therefore, according to the literature consulted, the individual differences between treatments in transport time, waiting time and stunning to slaughter time, are probably not high enough to cause a major effect in the animal welfare and stress indicators measured in the present study. However, when all of these factors are considered together plus other variations in handling, facilities and conditions between the slaughterhouses, some stress indicators indeed varied between treatments.

4.2. Stress indicators of animals slaughtered in local vs industrial abattoirs

Higher plasma glucose concentration could be biologically associated with an increase in catecholamines due to acute stress situations, which would use fast energy reserves in tissues such as glycogen to generate glucose. This rise would be reflected in the bloodstream (Tarrant, 1989). Differences in *post-mortem* glucose values have been related to different pre-slaughter practices such as transport (Tadich et al., 2003), waiting time at slaughterhouse and animal handling (Pighin et al., 2015). Despite all previous slaughter conditions from both treatments carried out following European well-being and animal welfare practices, animals slaughtered at IND abattoir may underwent some more stressing situations than LOC animals when considered the longer transport, the longer waiting time with other unknown animals in the waiting pens next door and, in addition, the superior stunning to bleeding time. Rumsey et al. (1999) observed through the Yellow Spring Instrument (YSI) that glucose values in beef cattle were between 60-110 mg/dL. In the present work, glucose values of LOC animals were slightly higher than the highest value of this interval (+6%), nevertheless, IND animals presented larger values (+39%) than these physiological values. Therefore, all the differences in pre-slaughter conditions between both treatments might explain the higher glucose levels observed in IND compared to LOC treatment at exsanguination.

Less glucose in the muscle would be available to form lactic acid (Immonem and Puolanne, 2000). This is why *longissimus* pH_{24h} was higher in IND than in LOC animals. However, in our study, no animal exceeded a pH of 5.8 at 24 h, and hence carcasses were not classified as DFD and they were within the commercially acceptable pH range. Nevertheless, five animals out of eight reached values over 5.7 in IND, while there were none in LOC. When pH was measured at 1 h, opposite results were found, with higher values in LOC *vs* IND, which means that a different rate of pH decline occurred. This would support the statement that early measures of pH are neither necessarily linked to pH values at 24 h nor to levels of glycogen in muscle (Young et al., 2004). Considering the disparities in the temperature conditions of dressing rooms and the time the carcasses were kept there before being placed in the cold room, these were high enough to probably impact on carcass temperature and pH decline (Marsh et al., 1981; Thompson, 2002).

Previous studies found that the duration of transport and the interval from stunning to bleeding may influence glucose levels and also the immunity status of animals, increasing the number of white cells (neutrophils) and decreasing the number of lymphocytes and their functions (Murata et al., 1987). In the present work, the neutrophil/lymphocyte ratio was threefold higher in IND *vs* LOC animals, which may be related to greater exposure to *pre-mortem* stress situations (Buckham Sporer et al., 2007) and a slight degree of associated immunosuppression (Anderson et al., 1999).

With regard to red cells, only mean corpuscular volume (MCV) was significantly higher in IND *vs* LOC animals. Red cells have been related to dehydration during long transport (Bernardini et al., 2012) or to some acute stress (e.g. heat shock) situations (Cardoso et al., 2015). However, apparent dehydration in the animals in the present study could not have occurred since transport time was short in both treatments and the temperatures were not extreme (Brunel et al., 2018).

Other common stress indicators such as creatinine kinase, lactate, NEFAs or cortisol levels were similar between both groups. However, cortisol was high when compared with previous studies. Cortisol baseline level in farm condition is around 50-70 nmol/L in *Bos taurus* cattle (Zavy et al., 1992; Villarroel et al., 2003). At exsanguination at commercial slaughterhouses, cortisol has been reported to be around 120 nmol/L (Tume and Shaw, 1992; Villarroel et al., 2003), level surpassed by most animals in the present study. Therefore, there is room for improvement in the two studied treatments. The effects of chronic stress cannot be overlooked either. Furthermore, improving animal welfare does not only involve working to minimise pain and suffering; it was proposed to consider the inclusion of positive experiences for animals during the pre-slaughter period, such as pleasant smells and activities to move closer to a more humane slaughter process (Browning, 2020; Browning and Veit, 2020).

4.3. Carcass, meat quality and consumer liking of animals slaughtered in local vs industrial abattoirs

Since breed, gender, age at slaughter and feeding management were similar between the two slaughter groups, carcass weight and conformation were similar and they matched usual characteristics of yearling Pirenaica breed calves (Altarriba et al., 2009). No significant differences between treatments were observed in the chemical composition of the *semitendinosus* muscle in the two groups, but fatness carcass score was higher in the IND group, which may indicate the influence of the classification at the abattoir, even though this classification is based on a normalised scale (EU) 2017/1182). In this section, we will describe differences in the two main parameters determining meat quality and purchase decisions: meat colour and tenderness.

The colour of *semitendinosus* steaks was lighter, less red, yellow and saturated and had a greater hue angle in LOC compared to IND. This is likely to be distinguished by consumers since Δ E, accounting for overall changes in L*, a* and b* (Hernández et al., 2019) at 24 h after cutting the steaks (when blooming was completed) was 4.60 and therefore, it might influence purchase decisions (Realini et al., 2014). Lightness is usually lower in meat with a higher pH due to changes in the meat structure from stressed animals or slaughter after undertaking physical activity. But a* and b* usually also decrease (Abril et al., 2001; Purslow et al., 2020; Apple et al., 1995; Apple et al., 2005; Ponnampalam et al., 2020). Even though pH at 24 hours measured in the *longissimus* muscle was higher in IND *vs* LOC meat, final pH values at 4 days after slaughter measured in the *semitendinosus* muscle was similar in both groups. Therefore, other factors further than pH might be involved in explaining differences in colour between treatments, such as temperature and pH drop before the rigor (Farouk and Lovatt, 2000; Purslow et al., 2020) or

iron content (Ponnampalam et al., 2020). No interaction between treatment and display time was found and furthermore, the spectrophotometric index, R630-R580, was similar for both slaughterhouses, which suggests that the meat packed in overwrap for 10 days deteriorated at a similar rate in both slaughter groups, probably because age and feeding was similar in both groups (Guerrero et al., 2013). Colour deterioration, which occurs over time (display time) due to an increase in meta-myoglobin development, was revealed by the decrease in a* values and saturation and the increase in hue, as reported in previous studies (Insausti et al., 1999), which was evident after seven days of display in both abattoirs. R630-580 is also related to meat discolouration, and values below 12.5 have been considered unacceptable for consumers (Renerre and Mazuel, 1985), which were reached on day 10 of display in both treatments in our study.

Tenderness is one of the factors that most influences consumer decision-making (Boleman et al., 1997). Consequently positive experiences of consumers after eating tender meat will affect future beef purchase decisions, which is highly relevant to the meat industry (Banovic et al., 2012). Cooked meat shear force was lower in LOC compared to IND group, which might explain why consumers gave better palatability scores to the former group, especially in terms of tenderness.

The effect of animal stress on tenderness is usually attributed to high 'abnormal' pH values (Silva et al., 1999; Jeleníková et al., 2008). However, in our study, no DFD meat was detected and pH differences between experimental groups were found in the *longissimus* muscle measured at 24 h post-mortem, but not in the *semitendinosus* muscle (on which texture analyses were conducted) at 4 days *post-mortem*. Some recent studies have shown evidence, however, that even meat with 'normal' pH obtained better eating quality scores when animals were less stressed prior to slaughter (Loudon et al. 2019), but the underlying mechanisms are not yet understood. Another study concluded that *pre-mortem* acute stress was related to differences in WBSF, regardless of the final muscle pH values (Gruber et al., 2010). In our study, although some signs of animal stress were higher in IND, the differences were minimal and probably are not the main cause of the difference in tenderness found. Other variations previously mentioned between groups, such as pH drop pre-rigor and temperature after slaughter likely played a major role in affecting texture (May et al., 1992).

Although our experimental design and analysis did not allow to stablish direct relationships between individual pre, post and slaughter conditions and meat quality, the fact is that meat from the LOC abattoir presented better consumer's acceptability, particularly after 11 days of ageing of the meat.

5. Conclusion

Considering altogether the results from this study and previous publications, it can be concluded that although transport time, waiting time (from arrival at slaughterhouse to stunning) and stunning to slaughter times were different between animals slaughtered at a local abattoir *vs* at an industrial one, these differences were probably not great enough to be considered individually as relevant additional sources of stress. When taking into consideration all of these factors together plus other variations in handling, facilities and conditions, this might have led to higher glucose levels and lower immunity in heifers slaughtered at the industrial abattoir. Other acute stress indicators such as cortisol were similar between the two groups but high compared to previous studies, so further improvements in term of animal welfare are needed in both commercial systems.

The pH in the *longissimus* muscle at 24 h post-slaughter was higher in the industrial abattoir compared to the local one, but pH assessed at 4 days in the *semitendinosus* muscle was statistically no different, so the change in the colour of the raw meat and in Warner Bratzler shear force and consumer acceptability was thought to be caused by other factors, such as rate of pH and temperature decline post-slaughter, due to different conditions in the dressing rooms at each abattoir. However, we are aware that the low number of animals analysed conditions our results and we are cautious in their interpretation. The data presented serve as a starting point to support the necessary diversification of beef production systems to preserve family farms and small local slaughterhouses that provide important added value in terms of the quality (in a broad sense) of the product and its sustainability.

CRediT authorship contribution statement

Pablo Guarnido-López: Investigation, Formal analysis, Writing – original draft. **Virginia Celia Resconi:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **María del Mar Campo:** Conceptualization, Investigation, Supervision, Writing – review & editing. **Ana Guerrero:** Investigation, Writing – review & editing. **Gustavo Adolfo María:** Conceptualization, Writing – review & editing. **José Luis Olleta:** Funding acquisition, Investigation, Conceptualization, Writing – review & editing.

Conflicts of interest

Authors declare no conflict of interest that could inappropriately influence (bias) our work.

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