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Title page

Evaluation of rumen temperature as a novel indicator of meat quality: Rumen temperature and haematological indicators of stress during the pre-slaughter period as predictors of instrumental meat quality in bulls

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Abstract

The use of new technologies such as rumen temperature boluses, together with the collective assessment of an animal stress responses may have the potential to act as an indicator of meat quality. Therefore, the objective of this study was to evaluate rumen temperature as a novel indicator of meat quality, by investigating its relationship with welfare measures and instrumental meat quality. The study involved 42 Holstein bulls (15.8 ± 0.08 months of age), which were transported 42km to a commercial abattoir. Mean rumen temperature rose by 0.511°C ($P < 0.001$) during the pre-slaughter phase; peaking during lairage. In addition, cortisol, creatine kinase (CK) and lactate dehydrogenase (LDH) were significantly ($P < 0.001$) elevated at slaughter. Bulls with a greater rumen temperature during the pre-slaughter phase produced meat with significantly higher pH_{ult} . Pre-slaughter rumen temperature was positively associated with slaughter CK, slaughter cortisol, pH_{ult} , L^* and a^* . Thus, rumen temperature demonstrates the potential to be used both as a novel welfare indicator and predictor of meat quality.

Keywords: rumen temperature bolus; stress-induced hyperthermia; ultimate pH; cortisol; creatine kinase

1.1 Introduction

Cattle are subject to a number of stressful situations; including transportation, social mixing and exposure to novel environments and personnel in the pre-slaughter period of their life (Ferguson & Warner, 2008; Miranda-de la Lama, Villarroel, & Maria, 2014). This period which consists of all events from loading at the farm until slaughter at the abattoir represents a risk to both animal welfare and meat quality (Hemsworth et al., 2011; Lomiwes, Farouk, Wu, & Young, 2014) which results in a significant cost for the red meat industry (Teke, Akdag, Ekiz, & Ugurlu, 2014). Suboptimal

meat quality is primarily caused by accelerated glycogenolysis during the pre-slaughter period. This process is initiated by the release of catecholamines following the activation of the sympathetic nervous system and is aimed at generating energy required for the initiation of a stress response (Ponnampalam et al., 2017). As a result meat quality is compromised due to insufficient acidification post-slaughter, which leads to a high ultimate pH (pH_{ult}) (Tarrant & Lacourt, 1984). Muscle activity, injury and fatigue can also be associated with a stress response, and will further add to the negative impact on meat quality. The occurrence of such muscular damage can be detected by the presence of muscle enzymes in serum such as creatine kinase (CK) and lactate dehydrogenase (LDH) (Losada-Espinosa, Villarroel, Maria, & Miranda-de la Lama, 2018).

Other haematological measures of a stress response are often used as welfare indicators; with the stress hormone cortisol being commonly used (Cafazzo et al., 2012). Cortisol is secreted in response to the release of adrenocorticotrophic hormone (ACTH) (Shaw & Tume, 1992); with changes in serum concentration being detectable minutes after exposure to a stressor, and lasting for up to two hours (Trunkfield & Broom, 1990).

Stress-induced hyperthermia is a well-documented physiological response to stressful events (Bouwknicht, Olivier, & Paylor, 2007; Losada-Espinosa et al., 2018; Pascual-Alonso et al., 2017; Pedernera-Romano, Ruiz de la Torre, Badiella, & Manteca, 2011). Hyperthermia occurs when the thermoregulatory system is altered by the autonomic nervous system; causing the thermoregulatory set point to be increased (Spooren, Schoeffter, Gasparini, Kuhn, & Gentsch, 2002). Rumen temperature boluses are a new technology primarily marketed for the detection of ill health, and they enable non-invasive, continuous data collection (Timsit, Assié, Quiniou, Seegers, & Bareille, 2011). Therefore, their use may assist with providing broader insights into stress-induced hyperthermia in cattle, particularly with regard to the effects of the handling, journey and lairage during the pre-slaughter period; an area where their application has so far been overlooked.

Thus, an observational study was conducted to determine associations between instrumental meat quality and the pre-slaughter phase rumen temperature and existing physiological welfare measures. Specifically, we examine the hypothesis that changes in rumen temperature will be associated with behavioural, haematological and meat quality indicators.

1.2 Materials and methods

This study was undertaken in June 2018 at the Agri-Food and Biosciences Institute (AFBI), Hillsborough, Northern Ireland. AFBI is located at latitudes and longitudes of 54.45° and -6.07°, respectively and is 91m above sea level. The area has a mean annual temperature of 8.6°C and a mean annual rainfall of 1024mm (Climate Data, 2019). Mean temperature for June 2018 was 15.1°C (MetOffice, 2018). All experimental procedures used in this study were conducted in compliance with the United Kingdom Animals (Scientific Procedures) Act 1986.

1.2.1 Animal management and experimental design

A total of 42 Holstein bulls, 15.8 ± 0.08 months of age and 575.9 ± 8.84 kg live weight were used in this study. Bulls were selected as they represent 10% of the prime cattle slaughtered in the UK, and are widely known to be more susceptible to stress than steers or heifers (Bonny et al., 2016; Tennessen, Price, & Berg, 1983). Bulls were housed indoors on slatted accommodation and split between 13 pens in groups of one ($n=1$), two ($n=8$), three ($n=21$) or four ($n=12$) bulls. Bulls were offered grass silage and concentrates *ad libitum* for 240 days prior to the commencement of this study, the chemical composition of these feeds is shown in Table 1. Bulls were transported to a commercial abattoir in a double decker lorry with passive ventilation, at a space allowance of 1.53m^2 . The journey which was primarily along a dual-carriageway was 42 km; with a journey time of 46 min. The lorry was subdivided into 8 pens and bulls were transported in a groups of four ($n=8$), five ($n=15$), six ($n=12$) or seven ($n=7$). Prior to unloading at the abattoir bulls had a stationary waiting time of 38 min. Bulls were all unloaded into one pen, and thus mixing occurred during lairage. Mean time spent in lairage was 86.9 min, and ranged from 56 to 117 min. The bulls were slaughtered using

the commercial abattoir procedure which consisted of stunning using the penetrative captive bolt method followed by exsanguination via ventral-neck incision. Electric goads were not used at any stage of this study, nor was electrical stimulation during processing.

Table 1: Chemical composition of feed

Chemical composition	Grass silage	Concentrates
DM (g kg ⁻¹)	272	866
CP (g kgDM ⁻¹)	124	150
ME (MJ kgDM ⁻¹)	10.6	11.1
ADF (g kgDM ⁻¹)	313	121
NDF (g kgDM ⁻¹)	532	295
Ash (g kgDM ⁻¹)	106	82.4
Nitrogen (g kgDM ⁻¹)	14.7	28.7

1.2.2 Environmental conditions

Farm conditions were recorded using a weather station (Davis Vantage Pro 2, Davis Instruments, USA) positioned externally in central point of the farm. Environmental conditions during transport were recorded using two iButtons (Hydrochron, DS1923 F5, Maxim Integrated, USA) which were secured to the sides of the lorry just above animal height; ensuring the face of the iButton was not obscured. In both cases ambient temperature (°C) and relative humidity (RH %) were recorded and used to calculate the temperature-humidity index (THI) for the farm and during transport. THI was calculated using the formula below where T is ambient temperature (°C) and RH is relative humidity expressed as a proportion (Hahn, Gaughan, Mader, & Eigenberg, 2009)

$$THI = 0.8T + RH(T - 14.4) + 46.4$$

1.2.3 Rumen temperature

Rumen temperature (°C) was automatically recorded at five minute intervals, using a rumen temperature bolus (ThermoBolus Small, Medria, France). The dimensions of the cylindrical bolus were 7x2.5cm, with a weight of 64.6g. The factory-calibrated bolus recorded to a tenth of a degree Celsius. Each bull had a rumen temperature bolus administered from three months of age; animals

were restrained in a head locking crush and boluses were administered orally using an applicator. Prior to transport, data was downloaded from the boluses continually, however once the bulls were loaded, the boluses were out of range for data download to occur. Thus, boluses were recovered from the rumen following slaughter and brought back to AFBI to allow the remaining data to be downloaded. A basal rumen temperature was established for each bull during the 48 h prior to loading. Rumen temperature during transportation and lairage were comprised of the durations between loading and unloading; and between unloading and slaughter, respectively. For each of the three phases, a mean and maximum rumen temperature were calculated.

1.2.4 Blood collection and live weight

Blood samples were taken at three time points; 24 h prior to transport (T1), 0 h prior to transport (T2) and at slaughter (T3). T1 and T2 were obtained via tail venipuncture at approximately the same time each morning; with the mean of these considered as an individual basal value. Bulls were also weighed full (due to an *ad libitum* diet) on both occasions using walk over scales; with the mean being considered as the slaughter weight. T3 was taken from the jugular vein during exsanguination at a mean of 249.2 ± 3.96 min (4 h 9 min) after T2. Blood (10ml) was collected into evacuated tubes (with no additive) at each time point for determination of cortisol, CK and LDH. Blood samples were stored at 4°C until centrifuged.

1.2.5 Observations

Stock person actions toward each animal were recorded during T1, T2 and at loading; in addition animal behaviour was also scored during loading. On each occasion bulls were identified by a number (1-42) which was spray painted across the animals back, this number was then cross-referenced with the bulls registered identification number. Ethograms and scoring systems (Table 2 and 3) were adapted from Hultgren et al. (2014); these ethograms were designed for use in a driving race at an abattoir and thus, minor alterations were made to each ethogram for application to this study. The observation period for T1 and T2 occurred for the full duration the animal was outside its

pen. A total of two trained stockpersons were present and scored; the first brought animals to and from the weigh area, the second moved the animals through the weigh area. Actions of the two stockpersons were combined at each time point to give scores for the full observational period. Two stockpersons were also present during loading, and the observational period was recorded using a GoPro Camera (Hero 5 Session), and videos later scored using The Observer XT13 software. Hultgren, Wiberg, Berg, Cvek, and Lunner Kolstrup (2014) also assigned an animal welfare rating (0=no effect, 1=mild, 2=moderate, 3=severe) for each action and behaviour; allowing the effect on animal welfare to be quantified. The sum of these scores during each observational period were used to develop an animal welfare rating for stockperson actions (AWR ACT) and an animal welfare rating for animal behaviour (AWR BEH) for each animal.

Table 2: Stockperson actions during handling and loading (adapted from Hultgren et al., 2014)

Action	Description	Animal Welfare rating
Speaking	Speaks or whistles softly and quietly	0
Shouting	Speaks or shouts harshly or loudly	2
Rattling	Makes noise by shakes rattle or clapping hands	1
Slamming	Makes noise by hitting wall with a tool	1
Touching rear	Touches the animal behind hip bone with hand	1
Touching front	Touches the animal in front of hip bone with hand	1
Slapping rear	Slaps the animal behind hip bone with hand	2
Slapping front	Slaps the animal in front of hip bone with hand	2
Touching rear -tool	Touches the animal behind hip bone with tool	1
Touching front -tool	Touches the animal in front of hip bone with tool	2
Hitting rear -tool	Hits the animal behind hip bone with tool	3
Hitting front -tool	Hits the animal in front of hip bone with tool	3
Booting	Kicks the animal	3
Gate hitting	Closes gate on animal	2
Tail twisting	Bends or twists tail	3
Any other action	Any other action	-

Table 3: Animal behaviours during loading (adapted from Hultgren et al., 2014)

Behaviour	Description	Animal Welfare rating
Crowding	Is prevented by other animals from moving forwards or backwards	2
Moving forwards	Moves at least 2 steps forwards	0
Moving backwards	Moves at least 2 steps backwards	2
Turning	Turns or attempts to turn	2
Running	Runs forward	2
Jumps onto lorry	Jumps onto ramp of lorry	0
Minor slipping	Feet slide, but continues to walk forward	1
Major slipping	Feet slide and stops walking, but only hooves or legs in contact with floor	2
Falling	Body in contact with floor	3
Kicking	Kicks legs behind towards stockperson	2
Minor struggling	Repeatedly trips, moves from side to side or kicks	2
Major struggling	Repeatedly throws itself against wall, jumps, tosses head, shivers or pants	3
Freezing	Stops moving and refuses to walk despite repeated attempts by stockperson	2
Vocalising	Makes vocal sounds	2
Exploring	Looks around or sniffs surrounding while standing still	0
Any other behaviour	Any other action	-

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153 1.2.6 Haematological variables

154 Blood samples were centrifuged at 3,000rpm at 4°C for 20 min. Plasma was collected from each
155 sample and stored at -20°C until analysis was conducted by NationWide Laboratories (England).
156 Cortisol concentration was determined using an Immulite 2000 analyser (Siemens Healthcare
157 Diagnostics Inc.,Belmont, CA) with a solid-phase competitive chemiluminescent enzyme
158 immunoassay. The mean interassay CV (n=20) was 5.0% for cortisol. CK and LDH were measured
159 using a clinical chemistry analyser (Model AU640; Beckman Coulter, UK). For CK the mean intraassay
160 CV (n=20) was 1.14% and the interassay CV (n=20) was 2.43%. Similarly, for LDH the mean intraassay
161 CV (n=20) was 1.13% and the interassay CV (n=20) was 1.52%.

162 1.2.7 Instrumental meat quality

163 At slaughter, carcass weight, fat classification and carcass conformation were recorded according to
164 the EU classification system. At 24 h post-slaughter, carcass sides were split between the 9th and 10th

rib, and the *longissimus dorsi* muscle was scored for marbling using the United States Department of Agriculture scoring system (Agricultural Research Council, 1965). Subcutaneous fat depth (mm) was also measured at three points; $\frac{1}{4}$, $\frac{1}{2}$ and $\frac{3}{4}$ along the length of the *longissimus dorsi* muscle on both sides of the carcass, the mean of these values was taken as the subcutaneous fat depth for each animal (Kirkland, Patterson, Keady, Moss, & Steen, 2007).

One meat sample (mean weight 0.93 ± 0.028 kg) from the *longissimus dorsi* muscle was removed from the fore-rib joint at 4 days post-slaughter. The meat sample was then split evenly into two samples which were aged at 3°C, one until D7 and the other until D14 post-slaughter. At D7 pH_{ult} was assessed using a Jenway 370 pH meter (Jenway, UK) and calibrated prior to measurements being taken using two buffer solutions of pH 4 and pH 7. Colour (L*(lightness), a*(redness) and b*(yellowness)) was also assessed on D7 using a Jenway 370 pH meter (Jenway, UK) which was calibrated using a white tile. D7 and D14 samples were then vacuum-packed and blast froze, where they were stored for two months until further measurements were taken.

Samples were left to thaw over a period of 24 h at 4 °C. Following this, they were removed from the packaging and left to bloom for 40 min. D7 and D14 samples were scored for marbling using the MSA scoring system (MLA, 2006); with the mean of D7 and D14 considered as the marbling score. Samples were vacuum-packed and cooked in a water bath at 70°C for 50 min. Samples were weighed pre- and post-cooking, and cooking loss was calculated as follows:

$$\text{Cooking loss \%} = \left(\frac{\text{pre cooking weight} - \text{post cooking weight}}{\text{pre cooking weight}} \right) 100$$

Warner Bratzler Shear Force (WBSF) measurements were completed on each sample using an Instron 3366 Universal Testing Instrument. Samples were cored parallel to the longitudinal orientation of the muscle fibres and 10 cores (sub-samples) were taken from each meat sample. All sub-samples were of the same diameter (12.5mm), and were sheared perpendicular to the muscle

fibres. The mean maximum load of the 10 sub-samples was considered as the WBSF value for each meat sample.

1.2.8 Statistical Analysis

All statistical analysis were completed using Genstat (16th edition). Summary statistics were conducted on carcass characteristics and animal welfare scores. Analysis of rumen temperature and haematological variables were carried out using linear mixed models with repeated measures. Animal was fitted as the subject factor and time point as a fixed effect. The correlation between time points was accounted for using an antedependence model of order 1. A series of one way ANOVAs with Fishers Protected LSD were used to examine the effect of pH_{ult} group on a range of measures including; rumen temperature, haematological variables, behavioural measures and meat quality. The models identifying factors associated with rumen temperature were developed using a stepwise multiple regression analysis, with both forward and backward selection methodologies employed. Explanatory variables included haematological variables (cortisol, CK and LDH) at slaughter (T3), and instrumental meat quality characteristics (pH_{ult}, L*,a*,b*, cooking loss and WBSF); combinations of these variables were compared to identify the best model. This model was then fitted to a multiple linear regression, and evaluated on the basis of residual variance. The models developed to predict meat quality characteristics were developed using the same methodology, however in this case the explanatory variables included haematological variables (cortisol, CK and LDH) at slaughter (T3), and rumen temperature.

1.3 Results

Two rumen temperature boluses were unable to be recovered at the abattoir and thus final temperature data was not obtained for these bulls. As a result, they were removed from the study; and the results presented below are for 40 bulls.

Mean ambient temperature and relative humidity ($\pm$ SEM) on the farm during the 24 hour period that this study was conducted were $12.88 \pm 0.241^{\circ}\text{C}$ and $78.76 \pm 1.260\%$, as a result THI was 55.34 ± 0.405 . Likewise, during transport mean values of were recorded $18.55 \pm 0.142^{\circ}\text{C}$ and $69.18 \pm 0.654\%$, subsequently, giving a mean THI of 64.13 ± 0.222 .

1.3.1 Observations

The mean of the animal welfare ratings derived from stock person actions during T1, T2 and loading, are shown in Table 4. Loading had the greatest mean animal welfare rating of 56.59, the mean number of times an animal was hit during loading was 4.2. This measure included four actions from the ethogram in Table 2; touches rear with tool, touches front with tool, hitting rear with tool and hitting front with tool. Mean animal welfare rating based on behaviour was 3.78; there were no incidences of falling, kicking or struggling during loading.

Table 4: Summary statistics of animal welfare ratings and hitting counts

	Mean	SEM	Median	Maximum
AWACT T1 ^a	8.95	1.552	6.5	40.0
AWACT T2 ^a	4.53	1.151	2.0	29.0
AWACT Loading ^a	56.59	9.055	29.8	229.5
Count of hitting during loading ^b	4.20	1.283	0.0	36.0
AWBEH Loading ^c	3.78	0.498	4.0	12.0

^a Sum of animal welfare rating per animal derived from stockperson actions
^b Count of hitting using a "tool" during loading
^c Sum of animal welfare scores per animal derived from behaviours

1.3.2 Rumen temperature

Basal: 48 hour period prior to loading; Transport: loading to unloading; Lairage: unloading to slaughter; a, b represent significant differences ($P<0.001$) between mean temperatures; x, y represent significant differences ($P<0.001$) between maximum temperatures.

Figure 1: Rumen temperature (°C) at three time points

Rumen temperature (Figure 1) increased significantly ($P<0.001$) during the pre-slaughter phase.

Mean temperature increased by 0.511°C , while maximum temperature increased by 0.568°C .

Although mean rumen temperature was not significantly different during transport or lairage;

maximum rumen temperature was significantly greater ($P<0.001$) during lairage ($40.46\pm0.085^{\circ}\text{C}$);

with 90% of bulls reaching their peak rumen temperature during this time.

1.3.3 Haematological variables

The effect of the pre-slaughter phase on haematological variables is presented in Table 5. Cortisol,

CK and LDH were all significantly elevated ($P<0.001$) at slaughter. Haematological variables did not

differ ($P>0.05$) between T1 and T2.

Table 5: Haematological variables of bulls at three timepoints

	T1	T2	T3	SED	F	dof	Sig.
Cortisol (nmol/l)	33.7 ^a	34.1 ^a	68.6 ^b	4.33	19.83	2/47.5	<0.001
CK (ui/l)	363 ^a	204 ^a	900 ^b	124.4	30.82	2/44.0	<0.001

LDH (ui/l)	3110 ^a	3141 ^a	3748 ^b	65.0	93.52	2/49.3	<0.001
T1: 24 h prior to transport; T2: 0 h prior to transport; T3: Slaughter. CK: creatine kinase; LDH: lactate dehydrogenase; a, b represent significant differences between timepoints.							

1.3.4 Carcass characteristics and meat quality

Table 6 outlines the carcass characteristics of the bulls used in this study. Carcass characteristics were not associated with rumen temperature, haematological variables or instrumental meat quality, and thus these relationships are not documented.

Table 6: Summary statistics of carcass characteristics

	Mean	SEM
Slaughter age (days)	479.6	2.24
Slaughter LW (kg)	576.0	8.73
Carcass weight (kg)	294.1	5.48
Carcass conformation ^a	4.35	0.141
Carcass fat classification ^b	7.05	0.232
Subcutaneous fat depth (mm)	6.47	0.277
Marbling (USDA) ^c	2.05	0.121
^a Score 1-15 (1=P-, 15=E+)		
^b Score 1-15 (1= 1-, 15=5+)		
^c Score 1-8 (1= A (Traces), 8=H (Abundant))		

When pH_{ult} is considered within three thresholds (<5.8; 5.8-6.2; >6.2) (Chulayo, Bradley, & Muchenje, 2016; Jeremiah, Tong, & Gibson, 1991) a clear difference is observed between rumen temperature values. Table 7 illustrates that bulls with a meat pH >6.2 had the greatest mean rumen temperature (40.13°C) during the pre-slaughter phase ($P<0.001$), in addition there was a tendency for mean rumen temperature to be increased when pH_{ult} was 5.8-6.2 in comparison to those <5.8. Maximum rumen temperature during the pre-slaughter phase was significantly different ($P<0.001$) within each of the three pH_{ult} ranges. There were no significant differences ($P>0.05$) in the duration that rumen temperature was elevated above the individual basal level between the three pH categories. In contrast, time spent above 40°C was significantly greater ($P<0.001$) for bulls with a pH_{ult} >6.2.

253 Stockperson actions, animal behaviour during loading and lairage duration did not differ ($P>0.05$)
 254 with pH_{ult}. Likewise, basal cortisol, CK and LDH did not differ with pH_{ult}, however, there was a
 255 tendency for basal levels of these haematological variables to be greater when pH_{ult} >6.2. Slaughter
 256 CK increased significantly ($P<0.001$) with increasing pH_{ult}.

257 There were no significant differences ($P>0.05$) in carcass characteristics between the three pH_{ult}
 258 ranges and thus these results are not shown in Table 7. Greater values for meat colour (L^* ($P<0.01$),
 259 a^* , b^* ($P<0.001$)) were observed for meat with a pH_{ult} <5.8. Cooking loss and WBSF were the lowest
 260 ($P<0.001$) for meat with a pH_{ult} >6.2. Although WBSF was numerically the greatest on day 7 and 14
 261 for a pH_{ult} range of 5.8-6.2, the difference was not significant from that at a pH_{ult} <5.8.

262 *1.3.5 Relationship between rumen temperature, haematological variables and instrumental meat*
 263 *quality*

264 Table 8 outlines the post-slaughter factors that were related to pre-slaughter phase rumen
 265 temperature. CK at slaughter was significantly ($P<0.001$) positively related to mean rumen
 266 temperature during transportation. The model for maximum lairage rumen temperature had the
 267 greatest R^2 value of 52.5 and included three variables: a^* , CK at slaughter and pH_{ult}. Mean rumen

Table 7: Rumen temperature, behavioural measures, haematological variables and instrumental meat quality according to ultimate pH

	Ultimate pH			SED	F	Sig.
	<5.8	5.8-6.2	>6.2			
Number of bulls (n)	19	11	10			
Basal RT (°C)	39.24	39.24	39.26	0.079	0.07	ns
Mean RT during transport (°C)	39.65 ^a	39.64 ^a	39.94 ^b	0.103	5.13	<0.05
Mean RT during lairage (°C)	39.55 ^a	39.63 ^a	40.43 ^b	0.262	6.46	<0.01
Maximum RT during basal period (°C)	39.83 ^a	39.80 ^a	40.25 ^b	0.143	5.64	<0.01
Maximum RT during transport (°C)	39.87 ^a	39.90 ^a	40.28 ^b	0.132	5.49	<0.01
Maximum RT during lairage (°C)	40.06 ^a	40.52 ^b	41.16 ^c	0.194	17.33	<0.001
Time RT > Basal (min)	190.0	175.0	185.0	11.64	0.99	ns
Time RT > 40°C (min)	47.1 ^a	58.2 ^a	125.0 ^b	19.76	8.64	<0.001
AWACT T1	6.8	7.1	15.0	3.78	2.76	ns

AWACT T2	4.9	3.3	5.3	2.99	0.23	ns
AWACT Loading	52	57	65	23.5	0.18	ns
Count of stockman hitting (Loading)	4	4	5	3.3	0.09	ns
AWBEH Loading	4	3	4	1.3	1.19	ns
Lairage duration (min)	93	54	79	7.5	1.93	ns
Basal Cortisol (nmol/l)	32.3	33.5	37.4	2.87	0.84	ns
Basal CK (ui/l)	161	162	653	239.0	2.56	ns
Basal LDH (ui/l)	3073	3076	3276	229.6	0.47	ns
Slaughter Cortisol (T3) (nmol/l)	62.1	76.5	72.1	14.2	0.65	ns
Slaughter CK (T3) (ui/l)	559 ^a	982 ^b	1458 ^c	211.0	9.79	<0.001
Slaughter LDH (T3) (ui/l)	3596	3829	3949	214.2	1.62	ns
L* (Lightness)	39.56 ^b	36.53 ^a	35.86 ^a	1.160	6.88	<0.01
a* (redness)	27.04 ^c	23.60 ^b	22.27 ^a	0.569	43.6	<0.001
b* (blue/yellow)	10.56 ^c	8.22 ^b	6.98 ^a	0.497	30.6	<0.001
Marbling Score (MSA)	718	682	722	50.1	0.37	ns
Cooking loss D7 (%)	26.05 ^c	23.63 ^b	19.58 ^a	0.737	40.9	<0.001
Cooking loss D14 (%)	25.68 ^b	24.99 ^b	19.43 ^a	1.381	12.1	<0.001
WBSF D7 (kg)	4.70 ^b	5.18 ^b	3.43 ^a	0.341	17.1	<0.001
WBSF D14 (kg)	4.46 ^b	4.71 ^b	2.96 ^a	0.266	22.1	<0.001

RT: Rumen temperature; AWACT: Sum of animal welfare scores per animal derived from stockperson actions; AWBEH: Sum of animal welfare scores per animal derived from animal behaviour; a,b,c represent significant differences between Ultimate pH threshold
ndf=2, ddf=37; n=40

temperature during transport was associated with CK at slaughter, while that during lairage was associated with CK and cortisol at slaughter. The model identified for maximum rumen temperature consisted of two meat quality parameters: pH_{ult} and L*.

Table 9 outlines the combination of haematological variables and rumen temperature measurements that can be used as predictors of meat quality. The greatest R² value (53.1) was observed for pH_{ult} with a combination of maximum lairage rumen temperature, CK and cortisol at slaughter being used as predictor variables. Maximum rumen temperature during lairage and CK at slaughter was the most common model for predicting meat quality; being used for three measures L*, a* and cooking loss D7.

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Table 8: Post-slaughter factors associated with rumen temperature

Y	Model (X ₁ +X ₂ +X ₃)	intercept	s.e.	t pr.	X ₁	s.e.	t pr.	X ₂	s.e.	t pr.	X ₃	s.e.	t pr.	R ²
Mean Transport RT	T3CK	39.52	0.0685	<0.001	0.00023	6.3E-05	<0.001							23.4
Mean Lairage RT	T3CK + T3Cort	39.81	0.241	<0.001	0.00061	0.00016	<0.001	-0.0081	0.0028	0.006				32.9
Maximum Transport RT	pH _{ult} + L*	35.78	1.44	<0.001	0.544	0.157	0.001	0.0247	0.0186	0.193				21.9
Maximum Lairage RT	a* + T3CK + pH _{ult}	39.69	2.99	<0.001	-0.0883	0.0501	0.087	0.0002	0.00015	0.133	0.467	0.335	0.172	52.5
Time RT>40°C	T3CK + pH _{ult}	-209.0	133.0	0.124	0.0396	0.0147	0.011	41.2	23.8	0.092				38.0
T3CK = creatine kinase at slaughter; T3Cort = cortisol at slaughter; pH _{ult} = ultimate pH; L* = lightness; a* = redness														

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Table 9: Prediction of meat quality based on rumen temperature and post-slaughter haematological variables

Y	Model (X ₁ +X ₂ +X ₃)	intercept	s.e.	t pr.	X ₁	s.e.	t pr.	X ₂	s.e.	t pr.	X ₃	s.e.	t pr.	R ²
pH _{ult}	MaxLRT + T3Cort + T3CK	-7.69	3.39	0.029	0.330	0.084	<0.001	0.0016	0.00129	0.220	0.00017	8.7E-05	1.950	53.1
L*	MaxLRT	120.5	30.2	<0.001	-2.045	0.747	0.009							14.3
a*	MaxLRT + T3CK	136.9	18.4	<0.001	-2.754	0.454	<0.001	-0.0086	0.0085	0.320				47.2
b*	MaxLRT + T3CK	67.40	17.6	<0.001	-1.422	0.441	0.003	-0.0009	0.00046	0.048				45.4
Cooking Loss D7	MaxLRT + T3CK	108.7	31.6	0.001	-2.072	0.793	0.013	-0.0013	0.00082	0.128				33.4
Cooking Loss D14	MaxLRT + T3Cort	112.1	41.2	0.010	-2.130	1.010	0.043	-0.0286	0.019	0.141				9.2
WBSF D7	MeanLRT + T3CK	25.00	10.0	0.017	-0.507	0.255	0.054	-0.0004	0.0003	0.203				17.5
WBSF D14	MeanLRT + T3CK	26.12	8.08	0.003	-0.546	0.205	0.011	-0.0003	0.00024	0.300				23.2
MaxLRT = maximum lairage rumen temperature; MeanLRT = mean lairage rumen temperature; T3CK = creatine kinase at slaughter; T3Cort = cortisol at slaughter														

1.4 Discussion

Consistent with our hypothesis, the results demonstrate that the continuous monitoring of rumen temperature during the pre-slaughter phase offers a tool to monitor aspects of animal welfare and meat quality, as evidenced by the relationships between these parameters that were uncovered by the current study.

1.4.1 Rumen temperature

Cattle exposed to heat stress will have a greater body temperature and respiration rate (Gaughan & Mader, 2014). However, in this study mean THI during transportation did not exceed the threshold of 65 outlined by Ammer, Lambertz, and Gauly (2016); above which a rise in rumen temperature can be expected. Thus, the observed rise in rumen temperature was not caused by heat stress. The initiation of a fight or flight response is controlled by the autonomic nervous system (ANS) resulting in a number of physiological changes. These are considered the first line of response upon exposure to a stressful situation, ensued by behavioural changes and the activation of the hypothalamic-pituitary-adrenal axis (HPA) (Losada-Espinosa et al., 2018). Thus, the elevated cortisol concentrations at slaughter and associations between rumen temperature and meat quality, indicate that the observed rise in rumen temperature during the pre-slaughter phase was caused by stress-induced hyperthermia.

Mean rumen temperatures during transport and lairage were similar, yet rumen temperature peaked higher during lairage, than in transportation; with bulls having a 0.48°C greater maximum temperature during lairage. Bulls had access to water during lairage, and when considering the findings of Bewley, Grott, Einstein, and Schutz (2008), who reported a reduction in rumen temperature associated with water intake; this could be the likely explanation as to why mean rumen temperature did not differ significantly during transport and lairage. However, it should be noted that bulls had access to water during the basal period, and yet mean basal rumen temperature and that of lairage, were significantly different. Thus, indicating that the rise in rumen

temperature due to stress-induced hyperthermia was substantial enough to supersede any decline caused by water intake.

Furthermore, the rise in rumen temperature during the pre-slaughter period indicates that both transport and lairage were considered novel environments. The progressive habituation of cattle to a novel environment has been well documented (Broom, 2003; Chacon, Garcia-Belenguer, Villarroel, & Maria, 2005; Njisane & Muchenje, 2017); with rectal temperatures of bulls peaking within the first 30 min of a 9 hour journey, before recovering to pre-transport levels (Burdick et al., 2010). However, in this study rumen temperature continued to rise throughout the pre-slaughter period, peaking during lairage. Therefore, as this study consisted of a short journey; it is likely that these bulls did not have sufficient time to acclimatise to transportation (Chacon et al., 2005). Hence, indicating that rumen temperature did not fully recover prior to exposure to a second novel environment; thus creating a cumulative stressor effect.

One further reason for the elevated temperatures during lairage was that bulls were largely transported within the groups they had been housed in. Whereas, at the abattoir they were all unloaded into one pen and thus, mixing occurred. Social mixing of bulls results in a greater occurrence of aggressive interactions as they attempt to establish a social hierarchy; thus creating social stress (Kenny & Tarrant, 1987; Tennessen, Pricea, & Berga, 1985; Warriss, Kestin, Brown, & Wilkins, 1984). Aggressiveness is also associated with increased physical effort, with possible carcass bruising and muscle damage; the extent of which will largely depend on the intensity and duration of such interactions (Ferguson & Warner, 2008). Therefore, mixing during lairage would have not only contributed towards the elevated rumen temperatures that were observed in this study; but also is the likely explanation for the elevated CK and LDH concentrations at slaughter (Losada-Espinosa et al., 2018; Tarrant, 1990). This is further supported by the findings shown in Table 8; where rumen temperature during lairage was associated with a^* , CK at slaughter and pH_{ult}. In addition Chacon et al. (2005) reported that transport had no effect on CK concentration, provided bulls were

transported under good conditions; with gentle handling and at recommended stocking densities.

Thus, muscle damage associated with transport could well have been minimal in this study.

1.4.2 Cortisol

Basal cortisol was in agreement with that of Buckham Sporer, Weber, Burton, Earley, and Crowe (2008); and Chacon et al. (2005), but greater than that observed by Cafazzo et al. (2012), and outlined as reference values by Radostits, Gay, Hinchcliff, and Constable (2007). Cortisol is widely known for its variability (Losada-Espinosa et al., 2018) with concentrations being impacted by numerous animal factors (Grandin, 2000); therefore, irregularity between studies is to be expected. Concentrations of cortisol doubled at slaughter (T3) in comparison to basal levels. Thus, indicating that the pre-slaughter environment was substantially more stressful than the routine handling associated with weighing and blood sampling; which may largely be due to the social mixing and novelty of the pre-slaughter environment (Bourguet, Deiss, Boissy, & Terlouw, 2015). Chacon et al. (2005) transported bulls for 3 different durations (30 min, 3 h & 6 h); the author observed greater cortisol concentrations for bulls with a journey time of 3 and 6 h in comparison to basal levels determined one week prior to transportation. Thus, indicating that a short journey (30 min) was not substantial enough to activate the hypothalamic-pituitary-adrenocortical axis to the same magnitude as a medium or long journey. Trunkfield and Broom (1990) outlined that a sharp and rapid increase was characteristic of cortisol; with rises occurring within minutes and lasting for up to 2 h. This raises the question as to which part of the pre-slaughter phase was responsible for the rise in cortisol detected in this study. It is possible that the bulk of the observed rise occurred during lairage and hence, cortisol may have followed the same pattern as rumen temperature. However, due to these cattle being slaughtered under commercial conditions, blood sampling post-transport was not practically feasible. Thus, the dynamic pattern of the haematological variables during the pre-slaughter phase of this study is unknown.

1.4.3 Instrumental meat quality

The high proportion of carcasses with a $pH_{ult} > 5.8$ are likely due to the individual's perception of the pre-slaughter environment and its associated stressors (Grandin, 1993). The cattle used in this study were naive to transportation, and were all managed within a small stable social group throughout their lifetime. This naivety to the stressors experienced during the pre-slaughter phase may have magnified the individual variability within the group, as individual stress responses were not impacted by previous habituation (Stockman et al., 2011).

Bulls with a $pH_{ult} > 6.2$ had a significantly greater maximum rumen temperature during the basal period; yet there was no difference in mean rumen temperature during this time. Thus, suggesting that these elevations in temperature were acute and hence causes of hyperthermia, such as ill-health could be eliminated (Timsit et al., 2011). Burdick et al. (2010) reported that temperamental bulls had a higher maximum rectal temperature than calm bulls during transportation. Furthermore, research has shown that temperamental or excitable cattle have a greater pH_{ult} , and poorer meat quality (Hall et al., 2011; Ponnampalam et al., 2017; Voisin et al., Grandin, O'Connor, Tatum, & Deesing, 1997); due to their increased susceptibility to stress. Therefore, in the current study, an increased maximum basal temperature may indicate that these bulls were more easily raised or stressed, even within an environment in which they were acclimatised to. In addition, the observed relationship between pH_{ult} and maximum temperature during both transportation and lairage, further supports this suggestion.

The cattle used in this study, were weighed fortnightly throughout their lifetime and daily feeding was also carried out by a stockperson. Consequently, they were accustomed to regular human interaction; which may be justification as to why stockperson actions had no impact on rumen temperature or meat quality. These findings are supported by previous research which observed a reduction in cortisol response as cattle became habituated to repeated handling (Andrade, Orihuela, Solano, & Galina, 2001; Solano, Galindo, Orihuela, & Galina, 2004). Waynert, Stookey, Schwartzkopf-

Genswein, Watts, and Waltz (1999) observed similar results when evaluating the impact of a stressor over time; with decreased heart rate and movement.

Rumen temperature during the pre-slaughter phase is associated with validated indicators of stress as shown by the models in Table 8. This is as expected, considering that stress results in a number of physiological changes while also initiating glycogenolysis, thus impacting meat quality (Ponnampalam et al., 2017). This demonstrates the potential for rumen temperature, measured using a bolus, to be used to provide broader insights into the welfare of ruminants. Furthermore, rumen temperature may also have the capacity to act as a pre-slaughter indicator of meat quality. When combined with haematological variables at slaughter, particularly CK and Cortisol, models can be identified to define the relationship with instrumental meat quality. This provides valuable information for the red meat industry, which may allow for preventative action to be taken prior to slaughter. Further research would be required to identify the extent to which animal factors (e.g. sex, age, breed) contribute to individual variability; so that a reliable threshold can be identified. In addition, remedial treatments could be evaluated in order to identify the rate at which rumen temperature can be reduced to acceptable levels prior to slaughter. A potential limitation of using rumen temperature boluses, may be the ability for them to be recovered post-slaughter. Although the boluses were easily recovered in this study, the practicalities are largely dependent on the abattoir and economics of recovery. Furthermore, the risk of bolus regurgitation should also be taken into consideration.

1.5 Conclusion

In conclusion, bulls had a significantly elevated mean rumen temperature during transport and lairage in comparison to basal levels. Rumen temperature peaked during lairage, possibly due to the cumulative effect of multiple stressors, and social stress associated with mixing during lairage. As a result pre-slaughter rumen temperature was significantly associated with pH_{ult}. Cortisol, CK and LDH were all significantly elevated at slaughter, however, CK was the only haematological variable that

increased as pH_{ult} increased. Therefore, through the continuous, non-invasive monitoring of stress induced hyperthermia in ruminants, a greater understanding of stress responses can be gained. Thus, rumen temperature boluses have the potential to be widely used as a welfare indicator and may even have uses as a monitoring tool within animal welfare monitoring schemes. In addition with further research, rumen temperature during the pre-slaughter phase may have the ability to act as a predictor of instrumental meat quality.

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