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A comparison of blood loss during the Halal slaughter of lambs following Traditional Religious Slaughter without stunning, Electric Head-Only Stunning and Post-Cut Electric Head-Only Stunning



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ABSTRACT

Blood lost at exsanguination during the Halal slaughter of lambs was compared between the slaughter methods of Traditional Religious Slaughter without stunning (TRS), Electric Head-Only Stunning (EHOS) and Post-Cut Electric Head-Only Stunning (PCEHOS). Two protocols were examined, Experimental (80 lambs) and Commercial (360 lambs), assessing varying periods of animal orientation during the 4 min bleeding process (upright orientation before vertical hanging). Live-weight, blood weight (Experimental only), carcass weights and by-product weights were recorded. The Experimental protocol highlighted an increase in blood loss at 60 s in EHOS and PCEHOS compared to TRS ($P < 0.001$) but by 90 s there was no significant difference. A post-slaughter change in animal orientation from an upright to a vertical hanging position aided the amount of blood loss. The bleeding of lambs is largely completed by 2 min. There were no significant differences ($P > 0.05$) in final blood loss between treatments. This research was undertaken to inform discussion on the merits of different slaughter methods compatible with Halal requirements.

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1. Introduction

The global value of trade in Halal and Kosher meat is significant with Muslim countries alone consuming meat estimated to be worth USD 57.2 bn in 2008 (Farouk, 2013). For Muslims, consuming only food that is Halal (meaning permissible) is a religious obligation. Riaz and Chaudry (2004, p8) stated the general principle: 'All foods are Halal except those that are specifically mentioned as Haram' (by the Qur'an (632) and the Hadiths as interpreted by Islamic Jurists). This basic principle was further outlined by Masri (2007, p134) when discussing 'lawful' and 'unlawful' meat. It is derived from the Qur'an (5:1, 6:145) and various Hadiths (Prophetic teachings) on the nature of food prohibitions. However many jurists have advised Muslim consumers to be cautious, particularly in relation to meat production (which must follow specific Islamic rules) in non-Muslim societies, by ruling all types of meat and animals to be Haram unless proven Halal (Taqi Usmani, 2006, p67). Masri (2007) stresses the great emphasis that Islam places on animal welfare during an animal's life and its slaughter for meat. For meat production Traditional Religious Slaughter without stunning (TRS) has been considered by the majority of Muslims as having the highest spiritual quality (Farouk et al., 2014; HMC, 2014) since the method was practiced by Prophet Muhammad and the earlier biblical

prophets (pbut). This reasoning also resonates with Jewish consumers in the production of Kosher meat.

During the last half-century as slaughter with stunning became more widespread some Muslims raised doubts over stunning methods (Nakyinsige et al., 2013) due to the potential violation of fundamental Halal criteria (Farouk, 2013) i.e., the animal must be alive (with certainty) at the point of slaughter, blood must not be consumed and the animal should not suffer unnecessarily during its life or slaughter. Since the consumption of blood is forbidden (Qur'an 2:173, 5:3, 6:145, 16:115) any process that expels maximum blood during the slaughter process would be favoured by Islamic scholars. Many Halal consumer groups are concerned about the effect of stunning on exsanguination and in particular there is a concern about a possible reduction in blood loss following the pre-slaughter stun or post-cut stun compared with a traditional cut alone (HMC, 2014; Nakyinsige et al., 2013). The focus of this paper is to compare blood loss at exsanguination following different methods of Halal slaughter. The issue of potential pain during slaughter is outside the scope of the research.

Muslim consumers regularly highlight their preference for TRS (EBLEX, 2010; HMC, 2014). However Electric Head-Only Stunning (EHOS) of sheep is accepted by many Muslim representatives as compatible with Halal criteria (ECFR, 1999; Farouk et al., 2014; IHIA, 2010; Nakyinsige et al., 2013) primarily because it is not known to result in the death of any sheep. As far as the authors are aware, there are neither studies (Salmano et al., 2013; Velarde et al., 2002) nor known

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incidences in industry where sheep have died as a result of EHOS. It is worth mentioning that many Muslims perceive stunning in general to be more painful for the animal (EBLEX, 2010, p17) particularly because of the occurrence of miss-stuns. Zivotofsky and Strous (2012) commented that electrical stunning 'should not be perceived as the panacea to animal welfare concerns in animal slaughter'. However, the occurrence of miss-stuns has been considerably reduced over the years and continuous assessment of the effectiveness of stunning is now a legal requirement across Europe (EC Regulation, 1099/2009).

A further potential Halal slaughter method is that of a Post-Cut Electric Head-Only Stunning (PCEHOS) (Farouk, 2013; Salamano et al., 2013). This option has the benefit that Muslim representatives know, without any element of doubt, that the animal is alive at the point of bleeding whilst also minimising one of the greatest potential welfare concerns, i.e., the length of time the animal is conscious after the cut and therefore the time it could potentially be experiencing pain (Gregory, 2005). It should be noted that the 'quality' of the slaughter period is arguably more important than the length of time to loss of consciousness because an extended time in which the animal is losing consciousness with minimum pain and distress is probably more desirable than a shorter more stressful period (Regenstein, personal communication). An additional advantage is that blood splash, a common complaint of Muslim consumers (Farouk et al., 2014), which can occur in EHOS occurs significantly less in PCEHOS (Kirton, Bishop, Mullord, & Frazerhurst, 1978).

Anil et al. (2004), Velarde, Gispert, Diestre, and Manteca (2003) and Warriss and Leach (1978) have each measured the bleeding of slaughtered lambs. Anil et al. (2004) slaughtered lambs in the prone position on a table before hoisting and collecting the blood. Velarde et al. (2003) hoisted conscious lamb before TRS, a practice now prohibited in Europe by an EC Regulation (1099/2009, Article 15(3)(a)). Warriss and Leach (1978) recorded blood loss from sheep slaughtered lying on their back on a cradle in comparison to those hung vertically on an overhead bleed line. It is known that the orientation of the animal at slaughter can affect blood loss (Velarde et al., 2014). Previous studies (Anil et al., 2004; Velarde et al., 2003; Warriss & Leach, 1978) did not assess blood loss in lambs slaughtered upright in a v-restraint and did not include PCEHOS as a potential Halal slaughter method. The objective of this study is to determine differences in blood loss at exsanguination in lambs following the slaughter methods of TRS, EHOS and PCEHOS when slaughtered upright in a v-restraint, as is currently common commercial practice in many Western countries. Residual blood content in the carcass was not assessed in these experiments as it is the blood loss at exsanguination rather than the residual blood that is of paramount importance for Halal slaughter (Farouk et al., 2014; Masri, 2007, p145). This research is expected to contribute to the development of Halal-compatible slaughter methods, animal welfare and to recommend further areas of study.

2. Materials and methods

This research compared legally permitted methods of slaughter in the UK and was approved by the University of Bristol, UK ethics committee (UIN number is UB/11/022). It was conducted in the UK in compliance with all requirements for such experiments. In total 440 lambs were assessed during the month of July.

A detailed literature review was conducted in preparation for the study and is reported elsewhere (HalalFocus, 2011). It is crucial for such research that religious stakeholders are engaged. Therefore, Islamic scholars and Halal stakeholders were invited to and attended the first day of the study. They were kept abreast of the experimental design and appraised the content of the associated literature review. Video was used to capture the main experimental procedures and key sequences are included in the online version of this paper.

The lambs were slaughtered using 3 different treatment groups (TRS, EHOS and PCEHOS) and subjected to 2 distinct handling protocols

categorised as Experimental or Commercial. During the Experimental protocol all animals were slaughtered upright in the v-restraint and held for 120 s before release; thus ensuring that every animal was bled using an identical orientation (note: this protocol is not practiced commercially in the UK). In contrast, during the Commercial protocol, animals were slaughtered under normal UK commercial practices (i.e., upright for TRS before being released and in dorsal recumbency for EHOS & PCEHOS before vertical hanging). Current UK legislation stipulates that TRS and PCEHOS lambs must be held for a minimum of 20 s in the restraint before release, whilst there are no holding restrictions for EHOS lambs. However, EC Regulation (1099/2009) does not stipulate any specific time for a holding period beyond the animal's loss of consciousness.

2.1. Animals

For the Experimental protocol (defined in 2.3.3.4) lambs were specifically sourced from a local farm within a 20-mile radius of the slaughterhouse in 2 groups. Group 1 comprised 40 males (38.2 kg \pm 1.3 live-weight at slaughter) with Group 2 comprising 40 females (37.8 kg \pm 1.1). All were Charollais-cross lambs from the same flock and of the same age (approx. 3.5 months old). Their necks were shorn on-farm and they were delivered to the abattoir using the farmer's own sheep trailer. Shearing the neck is not standard practice and was performed to minimize the possibility of blood flowing from the severed blood vessels being held in the neck wool.

Three different groups of lambs were sourced for the Commercial protocol (defined in 2.3.3.5). Group 3 consisted of 120 lambs (36.9 kg \pm 1.7) from a local farm within a 20-mile radius of the slaughterhouse and were transported using the farmer's own sheep trailer. Groups 4 and 5 comprised 150 lambs (38.5 kg \pm 2.8) and 90 lambs (38.2 kg \pm 2.5), respectively, and were bought from UK livestock markets within a 90-mile radius of the slaughterhouse and transported using commercial livestock hauliers. In order to replicate standard commercial practice none of the Commercial protocol lamb necks were shorn.

All animals arrived at the slaughterhouse at least 24 h before slaughter and no more than 48 h. Each group was kept in separate pens and rested, with ad-lib access to feed and water provided overnight. Click on multimedia file 1 for a video-clip of the animals in the slaughterhouse.

2.2. Equipment

Click on the multimedia files for videos (links below) and the 'supplementary file of images' for images of the main equipments used.

2.2.1. Live-weighing equipment

A factory calibrated live-animal weigher was used for the study, supplied by Morris Livestock Handling Equipment (Bishop's Castle, UK) and weighed lambs up to 100 kg in 0.1 kg increments. The digital weighing instrument calculated an average weight over time, to compensate for any lamb movement.

2.2.2. Restrainer

The lambs were restrained in a v-shaped rubber belt restraint supplied by Approved Design Ltd (Walsall, UK, www.adluk.net/machines/restraint.asp).

2.2.3. Slaughter knives

The knives best suited for the differing slaughter positions were selected. Two knives were used in total. Knife 1 was supplied by Spirit of Humane (Downing, WI, USA, <http://spiritofhumane.com/order>) and was used for all the upright slaughter of sheep in the v-restraint. It was 9 in. long and consisted of a straight blade similar to the Jewish Chalaf. Knife 2 was supplied from Dick (Deizisau, Germany, www.dick.de product number 8238510) and was used for the Commercial

protocol on the bleeding table following EHOS. It was 8.5 in. long. Click on multimedia file 2 for a video-clip of the knives.

2.2.4. Electric stunning equipment

The stunning equipment was comprised of the stun generator and the stun applicator. The stun generator was a model STUN-E514 stunner manufactured by Freund (Paderborn, Germany, www.freund-germany.com), which permits programmable control of all stunning parameters. The stun generator was purchased new and was less than 1 year old at the time of the experiments. The stun was applied using a Jarvis unit Model 2A handset, with an integral water spray, configured for head-only stunning (Jarvis Engineering N.Z., www.jarvisengineering.com/electrical-stunning).

2.2.5. Vision Data Logger

The Vision Data Acquisition System by Nicolet Instrument Technologies (Madison, WI, USA) which was subsequently acquired by HBM (www.hbm.com/en/menu/products/measurement-electronics-software/high-speed-data-acquisition/gen3i/nicolet-vision-and-gen3i-a-comparison) was used to digitally record the stunning current and voltage profiles for Groups 1, 2 (Experimental) and 3 (Commercial) using a rate of 5,000 samples/s. Click on multimedia file 2 for a video-clip of the Nicolet device in action. A Metrix Electronics Ltd differential probe GE 8115 (Bramley, UK, www.metrix-electronics.com/data/CAT_2010_P44_Accessories_GB1005.pdf) was used to measure the stunning voltage and a Fluke i30s AC/DC Current Clamp (Norwich, UK, www.fluke.com/fluke/uken/accessories/Current-clamps/i30.htm?PID=56292) was used to sample the stunning current. The equipment was calibrated on a standard load of 330 Ω in the laboratory and in the slaughterhouse to verify the profiles recorded.

2.2.6. Blood scale

An electronic digital bench scale was used to weigh the blood lost at exsanguination in restrained animals in increments of 0.02 kg. It was positioned beneath the end of the restrainer with the weight display unit positioned to one side. A tray was placed on the scales to capture the blood and a remote-controlled video camera recorded the display unit over time.

2.2.7. Carcass hanging scales

The bleeding and dressed carcasses were weighed using 3 heavy-duty hanging scales model AOC5-50 manufactured by ATP Instrumentation (Ashby-de-la-Zouch, UK, www.atp-instrumentation.co.uk). The scales weighed up to 50 kg in 0.02 kg increments and were purchased new for the experimental trial, i.e., factory calibrated.

2.2.8. By-product weighing scale

The by-products from each carcass were weighed using an Avery Berkel bench scale model L226 and load cell T110 with an upper weight range of 60 kg in 0.02 kg increments (Smethwick, UK, www.averyberkel.com). The scales were UK Trading Standards stamped.

2.2.9. Test weights

Prior to the start of data collection the live-weigher, blood scale, carcass hanging scales and by-product weighing scale were all checked and verified using a 20 kg calibrated test-weight.

2.3. Procedures

2.3.1. General procedures

Three practice trials were conducted beforehand with the same key operatives to ensure that the protocols developed were robust. The lambs were slaughtered over 3 non-consecutive days in July 2011. The first day comprised the Experimental protocol (Groups 1 and 2) and Group 3 of the Commercial protocol. The second and third day comprised Group 4 and 5 of the Commercial protocol respectively. The

slaughterhouse facility was made exclusively available for the whole day for Groups 1, 2, 3 and 4 and Group 5 was allocated an entire afternoon's production. There were no commercial time pressures on the authors or staff to expedite the process.

There were 3 main working 'stations' – the Live-Weighing Station (LWS), the Slaughter Station (SS) and the Evisceration Station (ES). Lambs were processed in batches of 13/14 (Experimental) or 10 (Commercial) animals with each whole batch assigned to one of the slaughter treatments by staff at the SS. The order of the slaughter treatments was randomised such that the data collectors at the LWS and ES were not aware of the slaughter method for each batch. Hence the LWS and ES procedures were standardized for all batches. The personnel at each station referred timings to their own digital clock synchronized to GMT.

2.3.2. LWS: Live-Weighing Station

Each lamb within a batch was marked with an individual number 1–14 (or 1–10) using animal marker spray and weighed. Live-weight was recorded only after the animal had settled and was relatively still. Each batch was weighed three times pre-slaughter on the same scale, with the time and weight recorded. On completion the batch was moved to the motorized crowd pen and fed into the v-restrainer in batch and individual number order. Click on multimedia file 3 for a video-clip of the Live-Weighing Station.

2.3.3. SS: Slaughter Station

The TRS, EHOS and PCEHOS slaughter processes were intended to deploy best available protocols for each treatment.

2.3.3.1. Stunning and slaughter personnel. The slaughter-man had worked at the abattoir for over a year and was experienced with the 3 experimental treatments. Similarly the stunning-operator had worked in that position for over a year and was comfortable with both EHOS and PCEHOS. Both participated in all three practice trials beforehand.

2.3.3.2. Knives. Knives were manually sharpened at the beginning of each day and were washed, steered and sterilized before each animal was slaughtered. The sharpness of the knife was tested frequently using a simple 'paper test' which tested the sharpness of the blade on a sheet of A4 paper (Grandin, personal communication). The knives were re-sharpened if it did not slice the paper smoothly. Subjective visual assessment of the slaughter action confirmed a 'clean' cut with multiple correcting cuts (more than 2) assessed as a 'miss-cut'.

2.3.3.3. Stun parameters. The stunner output was pre-selected to deliver a 1.0 A constant current for 3 s at 200 Hz. 1.0 A is the minimum requirement for sheep under EC Regulation (1099/2009) and recommended guideline parameters for sheep as stated by the IHIA (2010) i.e., 1 A for 1–3 s (frequency was not specified). Water was applied to the lamb's heads pre-stun. The parameters were identical for EHOS and PCEHOS and were verified by the records obtained from the Vision Data Logger for Groups 1–3. Subjective visual assessments of the animal's head, eyes and legs confirmed effective stunning with multiple stun applications (more than 2) assessed as a 'miss-stun'.

2.3.3.4. Experimental protocol. Experimental lambs were held upright in the restrainer for 120 s to bleed regardless of their treatment. The cut was made at $t = 0$ s by manually lifting the lamb's head and performing a deep ventral neck cut, cutting the skin, muscle, carotid arteries, jugular veins, oesophagus, trachea and major nerves within approximately 5 s of the stun for the EHOS or PCEHOS treatments. Blood flowed from the wound, down the neck and was collected in the tray beneath the restrainer. The weight and rate of blood loss was displayed and recorded by video camera over the 120 s bleeding period. Following bleeding, the lambs were ejected, shackled and hung vertically on the hanging scale. Carcass weight was recorded at 3 and 4 min post-cut before the carcass was released for further processing. Lambs were slaughtered

every 3 min to facilitate the process flow. Click on multimedia files 4a, 4b and 4c for video-clips of all 3 treatments during the Experimental protocol.

2.3.3.5. Commercial protocol. Commercial lambs were processed in accordance with normal slaughtering procedures for the abattoir and existing UK legislation. Lambs were slaughtered every 2 min. The procedures that were adopted were as follows:

- EHOS: Lambs were stunned upright in the restrainer, ejected onto the table, slaughtered in a horizontal position (dorsal recumbency), shackled and hung vertically onto the hanging scale with carcass weight recorded at 1, 2, 3 and 4 min post-cut.
- TRS: Lambs were cut and ejected after 20 s (by which time all lambs were assessed as having lost consciousness). They were subsequently shackled and hung vertically onto the hanging scale. Carcass weight was recorded as for EHOS.
- PCEHOS: This was similar to TRS but the lambs were stunned within 5 s post cut.

Click on multimedia files 5a, 5b and 5c for video-clips of all 3 treatments during the Commercial protocol.

2.3.4. ES: Evisceration Station

Data were collected in a systematic way in the order that carcasses were processed to prevent loss of data integrity. There were nine categories of by-products: 'Stomach/Intestine' (S/I), 'Heads and Feet' (H/F), 'Skin', 'Liver', 'Lungs', 'Heart', 'Spleen', 'Gall Bladder' and 'Pluck Remainder'. Operators began dressing procedures near the end of the bleeding line. Laminated sheets numbered from 1–14 helped correlate H/F and Skin to the correct carcass. H/F were collected in one large plastic bag together with any other trimmings from the carcass (tail/wool/skin etc.). S/I included the full stomach and intestine. The 'Pluck Remainder' comprised any parts of the red offal left after the liver, heart and lungs were removed. Instances such as gut spills were recorded. By-products were weighed in designated trays on the Avery scale. Dressed Hot Carcasses were weighed on the hanging weigh-scale with weight and time recorded to establish the Processing Time (from final live-weighing to carcass weighing). Click on multimedia file 6 for a video-clip of the Evisceration Station.

2.4. Statistical analysis

Data were initially entered into Excel for integrity checks. Other variables such as Kill-Out Percentage (KO%) were calculated (part weight as a percentage of animal live-weight). After a considered assessment, data were rejected from further analysis if data integrity safe-guards (defined below) were outside acceptable limits.

The rejection criteria were as follows.

1. *Live-weight range* between the highest and lowest of the 3 repeated live-weights for each animal is greater than 0.5 kg.

2. *Miss-stuns* (more than 2 stuns) or *Miss-cuts* (more than 2 cuts) following visual observations on the day of the experiments. Note: Unusual stun profiles were not rejected.
3. *Unaccounted Weight (UA)* (after deducting blood, carcass and all measured by-products from the animal live-weight) was less than –0.2 kg or greater than 1 kg.
4. *Blood Loss % (BL%)* (blood loss/avg live-weight) rejected if the final BL% was less than 2.5% or greater than 7.5%. This has the effect of rejecting data showing a release of less than 1 kg or greater than 3 kg of blood, which from commercial experience appears reasonable.

Table 1 outlines the implications of the rejection criteria on data analysis. There were sufficient data remaining to perform detailed statistical analysis. The data were analysed using a General Linear Model with Live-weight as a covariate. The software package used was PASW Statistics 18 (www.spss.com.hk/statistics/). The statistical criteria for significance were $P < 0.05$.

The Vision data-logger was used to record the constant-current stun profiles for the three treatment groups. An analysis was later carried out in the laboratory and an assessment of each stun start and finish position was made. After reviewing the stun profiles they were subjectively categorised as 'Good', 'Wave modulated' and 'Unusual' based on the voltage profile. Fig. 1 and multimedia file 2 shows a typical 'Good' stun profile taken from an EHOS recording. The 'unusual' profiles are those where there was a break in contact between the electrodes and the animal (e.g., not continuous, abrupt finish, poor initial contact, etc.). The 'wave modulated' profiles were not as smooth as the 'Good' profiles but still appeared to produce the required current. The corresponding Volts Root Mean Square (RMS) from the Vision data-logger was recorded manually into Excel and subsequently analysed statistically using a General Linear Model with no covariate.

3. Results

Click on the Excel spreadsheet for the entire research data recorded. It should be noted that, with the exception of the 10 s values from the initial 120 s blood loss during the Experimental protocol (direct blood loss), all the remaining blood values were calculated as the difference between average live-weight and carcass weight during bleeding (indirect blood loss). Consequently indirect blood loss values will incorporate other liquids released from the animal, particularly rumen content. The terminology used to differentiate between direct and indirect blood loss data is '120 s blood' and '2-minute blood' respectively.

3.1. Experimental protocol data

Fig. 2 shows average blood loss for each treatment over the initial 120 s period whilst Table 2 presents the results of the statistical analysis of the Experimental protocol data. Live-weight was not significantly different between treatment groups ($P = 0.968$) and was used as a covariate within the analysis.

Table 1
Implications of rejection criteria on data analysis.

| Groups | Treat | Initial data | LW range >0.5 kg | Miss-stun | Miss-cut | Unacc weight <–0.2 kg | Unacc weight >1 kg | Final BL% <2.5% | Final BL% >7.5% | Insuff BL data | Final data for analysis |
|---------------------|--------|--------------|---------------------|-----------|----------|--------------------------|-----------------------|--------------------|--------------------|----------------|----------------------------|
| <i>Experimental</i> | | | | | | | | | | | |
| 1 & 2 | TRS | 27 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 1 | 22 |
| 1 & 2 | EHOS | 26 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 24 |
| 1 & 2 | PCEHOS | 27 | 0 | 1 | 0 | 6 | 0 | 0 | 0 | 0 | 20 |
| 1 & 2 | N | 80 | 0 | 1 | 0 | 10 | 2 | 0 | 0 | 1 | 66 |
| <i>Commercial</i> | | | | | | | | | | | |
| 3, 4 & 5 | TRS | 120 | 3 | 0 | 1 | 6 | 7 | 1 | 1 | 0 | 101 |
| 3, 4 & 5 | EHOS | 120 | 6 | 1 | 0 | 3 | 1 | 0 | 6 | 0 | 103 |
| 3, 4 & 5 | PCEHOS | 120 | 2 | 0 | 1 | 4 | 5 | 1 | 4 | 0 | 103 |
| 3, 4 & 5 | N | 360 | 11 | 1 | 2 | 13 | 13 | 2 | 11 | 0 | 307 |

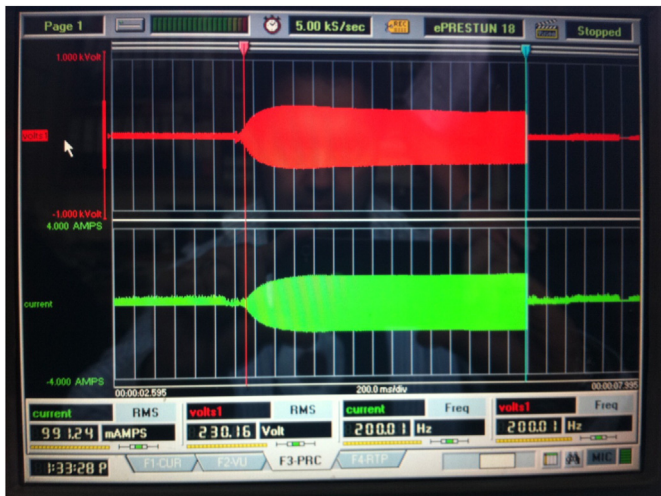


Fig. 1. Example of 'Good' EHOS stun profile on Vision Data Logger.

Nearly all the blood was lost within 90 s. The rate of blood loss was slowest in TRS after 10 s and, subsequently, blood loss at 60 s was significantly less ($P < 0.001$) in TRS compared with both EHOS and PCEHOS. However by 90 s there was no significant difference. The profile of PCEHOS closely followed EHOS.

The effect of gravity following the release of the carcass from the v-restrainer at 120 s (upright) onto the carcass hanging scale by one rear leg recorded at 3-minutes (hanging vertically) caused a large increase in blood loss, which potentially included rumen contents. There were no significant differences between treatments at 3-minute blood loss, 4-minute blood loss or 4-minute blood loss %.

S/I weights were highly variable as seen from the relatively large standard error values for S/I and KO%. S/I weight and KO% for PCEHOS was significantly greater ($P < 0.05$) than for TRS. PCEHOS took significantly more Processing Time than EHOS ($P < 0.05$) but this did not correlate with an increase in UA. The Gall Bladder weight in EHOS was significantly less than the TRS ($P < 0.05$) and PCEHOS ($P < 0.01$) treatment groups. UA was significantly higher in TRS than EHOS ($P < 0.01$) and PCEHOS ($P < 0.05$).

3.2. Commercial protocol data

Table 3 presents the results of key data analysis of the Commercial protocol by group. Only Group 3 produced significant differences in blood loss values. For 4-minute blood loss %, PCEHOS was significantly less than TRS ($P < 0.001$) and EHOS ($P < 0.05$) with the difference between TRS and EHOS tending towards significance ($P \sim 0.07$).

Group 3 PCEHOS skin weight was heavier than the TRS and EHOS treatments ($P < 0.05$) by over 0.2 kg on average. Lung weights across

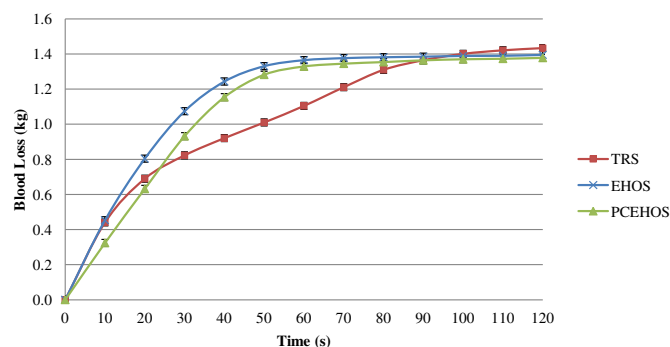


Fig. 2. Cumulated Mean Blood Loss (kg) \pm SE during initial 120 s of the Experimental protocol.

all three groups were highest in TRS, followed by PCEHOS and then EHOS but they were only significantly different in Groups 4 and 5. This finding should be treated with caution as no difference was observed during the Experimental protocol, when lambs were held upright for much longer (2 min). Group 5 live-weight was significantly lighter ($P < 0.05$) for PCEHOS compared to EHOS but after adding live-weight as a covariate in the statistical software this did not contribute to significant differences in the remaining key data analysed. There were no other significant differences.

Table 4 shows the results of the Commercial data combined. After accounting for the date effect, live-weight was not significant and was added as a covariate. The 1-minute blood loss was also not significant with its respective lower degrees of freedom relating to lower data collections due to unstable readings following post-slaughter carcass convulsions (clonic activity) in some lambs. Final blood loss value was significantly lower in PCEHOS ($P < 0.05$) compared to TRS or EHOS as a result of Group 3 but this was not seen in Group 4 or 5.

Lung weight was significantly higher in TRS ($P < 0.001$) than EHOS or PCEHOS. Pluck remainder weight was significantly lower in EHOS ($P < 0.01$) than TRS or PCEHOS. There were no significant differences in the remaining data.

3.3. Electrical stun profiles

The subjective categorization of voltage profiles are shown in Table 5. There were no observable differences between the respective profiles of EHOS and PCEHOS except that the initial contact was sometimes less efficient for PCEHOS possibly because the neck cut made the placement of the electrodes slightly more difficult for the operator. Five stuns were not recorded due to human error. To account for real-life stun variation no data were rejected from the analysis. There were no significant differences in voltage RMS between EHOS and PCEHOS with either the Experimental or Commercial protocols (Table 6).

4. Discussion

After an effective neck cut sheep generally lose consciousness (EEG assessment) within 2–7 s (Newhook & Blackmore, 1982) and cortical brain death occurs by approximately 14 s (Gregory & Wotton, 1984). In contrast, Rodriguez, Velarde, Dalmau, and Lonch (2012) found that the onset of unconsciousness in lambs could extend to 1 min and postulated that this difference from the results of other researchers could be due to potential inefficiencies in bleeding. The results from this research show that the majority of the blood is lost within the first 2 min and demonstrates that, in agreement with previous studies (Hopkins, Shaw, Baud, & Walker, 2006; Kirton, Frazerhurst, Woods, & Chrystall, 1981), the bleeding of lambs during exsanguination is completed within 2 min. Therefore, from a slaughterhouse perspective, there is no need to allow additional bleed time to increase blood loss.

4.1. Experimental protocol

EHOS is known to increase blood pressure by an average of 3.5 times within an average 11 s from the stun (Kirton et al., 1978). One of the actions of EHOS is cardiac inhibition via parasympathetic stimulation mediated by the vagus nerve. Once the stun application is removed, baro-receptors (blood pressure monitors) in the major blood vessels register a low blood pressure and the heart rate increases, which produces an overshoot in heart rate and subsequently blood pressure (Leach & Warrington, 1976). This overshoot in blood pressure would affect the rate of blood loss until diastolic pressure failed due to exsanguination. Consequently it seems reasonable that EHOS, and by extension PCEHOS, should lose blood more rapidly in the initial phases compared to TRS. Anil et al. (2004) produced a similar profile of blood loss during exsanguination and concluded there was no significant difference in blood loss between TRS and EHOS. The 90 s blood loss values were not

Table 2
Experimental protocol data analysis with treatment as a fixed factor.

| Treatment | TRS | | EHOS | | PCEHOS | | df | F | Sig |
|----------------------|---------------------|-------|---------------------|-------|--------------------|-------|----|-------|-------|
| | Mean | SE | Mean | SE | Mean | SE | | | |
| N | 22 | | 24 | | 20 | | | | |
| Live-weight (kg) | 38.0 | 0.3 | 38.0 | 0.3 | 38.0 | 0.3 | 63 | 0.03 | ns |
| 60 s blood (kg) | 1.10 ^b | 0.03 | 1.37 ^a | 0.03 | 1.33 ^a | 0.03 | 62 | 19.42 | *** |
| 90 s blood (kg) | 1.36 | 0.03 | 1.39 | 0.03 | 1.37 | 0.03 | 62 | 0.17 | ns |
| 120 s blood (kg) | 1.43 | 0.03 | 1.40 | 0.03 | 1.38 | 0.03 | 62 | 0.78 | ns |
| 3 min blood (kg) | 1.95 | 0.06 | 1.93 | 0.06 | 1.90 | 0.06 | 62 | 0.14 | ns |
| 4 min blood (kg) | 1.96 | 0.06 | 1.95 | 0.06 | 1.93 | 0.06 | 62 | 0.09 | ns |
| 4 min blood % | 5.2% | 0.2% | 5.1% | 0.2% | 5.1% | 0.2% | 62 | 0.09 | ns |
| S/I (kg) | 6.81 ^a | 0.15 | 7.09 ^{ab} | 0.14 | 7.24 ^b | 0.15 | 62 | 2.13 | * |
| S/I KO% | 17.9% ^a | 0.4% | 18.7% ^{ab} | 0.4% | 19.1% ^b | 0.4% | 62 | 2.17 | * |
| Hot carcass (kg) | 20.78 | 0.16 | 20.75 | 0.16 | 20.44 | 0.17 | 62 | 1.19 | ns |
| Hot carcass KO% | 54.7% | 0.4% | 54.6% | 0.4% | 53.8% | 0.5% | 62 | 1.22 | ns |
| Processing Time | 01:25 ^{ab} | 00:04 | 01:19 ^a | 00:04 | 01:35 ^b | 00:04 | 62 | 2.75 | * |
| Heads & feet (kg) | 3.56 | 0.03 | 3.52 | 0.03 | 3.54 | 0.04 | 62 | 0.43 | ns |
| Skin (kg) | 3.02 | 0.06 | 2.96 | 0.06 | 3.11 | 0.06 | 62 | 1.52 | ns |
| Liver (kg) | 0.704 | 0.014 | 0.708 | 0.013 | 0.711 | 0.014 | 62 | 0.07 | ns |
| Lungs (kg) | 0.419 | 0.009 | 0.438 | 0.008 | 0.416 | 0.009 | 62 | 1.91 | ns |
| Heart (kg) | 0.168 | 0.004 | 0.175 | 0.004 | 0.171 | 0.004 | 62 | 1.00 | ns |
| Spleen (kg) | 0.069 | 0.002 | 0.070 | 0.002 | 0.068 | 0.002 | 62 | 0.19 | ns |
| Gall bladder (kg) | 0.028 ^b | 0.002 | 0.022 ^a | 0.002 | 0.030 ^b | 0.002 | 62 | 4.73 | *, ** |
| Pluck Remainder (kg) | 0.196 | 0.007 | 0.199 | 0.007 | 0.207 | 0.007 | 62 | 0.60 | ns |
| UA (kg) | 0.266 ^b | 0.040 | 0.102 ^a | 0.038 | 0.121 ^a | 0.042 | 62 | 5.12 | **, * |

Values in a row with different superscripts differ significantly.

df = degrees of freedom. F = variance ratio.

ns = not significant. Significance at * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Significance levels separated by a comma indicate respective levels of significance in the order of the columns TRS, EHOS, PCEHOS.

S/I = Stomach/Intestine, KO% (Kill-Out %) = percentage of that animal product over the live-weight, Processing Time = the time (in hours and minutes) between the last live-weight and the final carcass weight, Pluck Remainder = the remainder of the red offal or 'pluck', UA = Unaccounted Weight after deducting 4 min blood, carcass and all by-products from the live-weight.

significantly different between treatments in either publication and the average 90 s blood loss % were similar between treatments and between publications (this research: EHOS 3.65% and TRS 3.58%, Anil et al. (2004): 3.78% and 3.98% respectively.) Anil et al. (2004) found

significant differences in live-weight between treatments and had to adjust blood loss values as a result. It is not possible to compare blood loss data between publications directly as the adjusted live-weight data were not reported in the Anil et al. paper. The blood loss profiles

Table 3
Commercial protocol data analysis – key data by groups.

| Treatment | TRS | | EHOS | | PCEHOS | | df | F | Sig |
|------------------|--------------------|-------|--------------------|-------|--------------------|-------|-----|------|-------|
| | Mean | SE | Mean | SE | Mean | SE | | | |
| Group 3 (N) | 33 | | 36 | | 36 | | | | |
| Live-weight (kg) | 36.8 | 0.3 | 37.0 | 0.3 | 36.7 | 0.3 | 102 | 0.26 | ns |
| 4 min blood (kg) | 1.74 ^a | 0.04 | 1.63 ^a | 0.04 | 1.51 ^b | 0.04 | 101 | 7.60 | *** * |
| 4 min blood % | 4.7% ^a | 0.1% | 4.4% ^a | 0.1% | 4.1% ^b | 0.1% | 101 | 7.89 | *** |
| S/I (kg) | 7.08 | 0.13 | 7.09 | 0.12 | 6.89 | 0.12 | 101 | 0.91 | ns |
| S/I KO% | 19.2% | 0.3% | 19.2% | 0.3% | 18.7% | 0.3% | 101 | 0.86 | ns |
| Skin (kg) | 3.04 ^a | 0.07 | 3.05 ^a | 0.07 | 3.29 ^b | 0.07 | 101 | 4.56 | * |
| Lungs (kg) | 0.470 | 0.012 | 0.438 | 0.012 | 0.444 | 0.012 | 101 | 1.99 | ns |
| Group 4 (N) | 42 | | 41 | | 41 | | | | |
| Live-weight (kg) | 38.2 | 0.4 | 38.2 | 0.4 | 39.0 | 0.4 | 121 | 1.22 | ns |
| 4 min blood (kg) | 1.88 | 0.04 | 1.89 | 0.04 | 1.87 | 0.04 | 120 | 0.06 | ns |
| 4 min blood % | 4.9% | 0.1% | 4.9% | 0.1% | 4.9% | 0.1% | 120 | 0.08 | ns |
| S/I (kg) | 7.72 | 0.12 | 7.82 | 0.13 | 7.83 | 0.13 | 120 | 0.24 | ns |
| S/I KO% | 20.1% | 0.3% | 20.3% | 0.3% | 20.4% | 0.3% | 120 | 0.20 | ns |
| Skin (kg) | 3.20 | 0.05 | 3.16 | 0.05 | 3.19 | 0.05 | 120 | 0.16 | ns |
| Lungs (kg) | 0.461 ^b | 0.010 | 0.411 ^a | 0.011 | 0.416 ^a | 0.011 | 120 | 7.05 | *** |
| Group 5 (N) | 26 | | 26 | | 26 | | | | |
| Live-weight (kg) | 38.3 ^{ab} | 0.5 | 39.0 ^a | 0.5 | 37.6 ^b | 0.5 | 75 | 2.32 | * |
| 4 min blood (kg) | 1.98 | 0.05 | 2.02 | 0.05 | 1.96 | 0.05 | 74 | 0.30 | ns |
| 4 min blood % | 5.2% | 0.1% | 5.3% | 0.1% | 5.1% | 0.1% | 74 | 0.30 | ns |
| S/I (kg) | 7.59 | 0.17 | 7.56 | 0.17 | 7.44 | 0.17 | 74 | 0.21 | ns |
| S/I KO% | 19.8% | 0.4% | 19.7% | 0.5% | 19.4% | 0.5% | 74 | 0.21 | ns |
| Skin (kg) | 3.07 | 0.09 | 3.17 | 0.09 | 3.18 | 0.09 | 74 | 0.41 | ns |
| Lungs (kg) | 0.457 ^b | 0.012 | 0.401 ^a | 0.012 | 0.419 ^a | 0.012 | 74 | 5.56 | **, * |

Values in a row with different superscripts differ significantly.

df = degrees of freedom. F = variance ratio.

ns = not significant. Significance at * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Significance levels separated by a comma indicate respective levels of significance in the order of the columns TRS, EHOS, PCEHOS.

S/I = Stomach/Intestine, KO% (Kill-Out %) = percentage of that animal product over the live-weight.

Table 4

Commercial protocol data analysis – groups 3–5 combined with treatment and date as fixed factors.

| Treatment | TRS | | EHOS | | PCEHOS | | df | F | Sig |
|----------------------|--------------------|-------|--------------------|-------|--------------------|-------|-----|-------|-----|
| | Mean | SE | Mean | SE | Mean | SE | | | |
| N | 101 | | 103 | | 103 | | | | |
| Live-weight (kg) | 37.8 | 0.2 | 38.1 | 0.2 | 37.8 | 0.2 | 298 | 0.59 | ns |
| 1 min blood (kg) | 1.56 | 0.03 | 1.63 | 0.04 | 1.60 | 0.04 | 188 | 0.96 | ns |
| 2 min blood (kg) | 1.80 ^{ab} | 0.03 | 1.81 ^a | 0.03 | 1.73 ^b | 0.03 | 297 | 2.53 | * |
| 3 min blood (kg) | 1.85 ^a | 0.03 | 1.83 ^{ab} | 0.03 | 1.76 ^b | 0.03 | 297 | 3.07 | * |
| 4 min blood (kg) | 1.87 ^a | 0.03 | 1.85 ^{ab} | 0.03 | 1.78 ^b | 0.03 | 297 | 2.92 | * |
| 4 min blood % | 4.9% ^a | 0.1% | 4.9% ^{ab} | 0.1% | 4.7% ^b | 0.1% | 297 | 3.05 | * |
| S/I (kg) | 7.47 | 0.08 | 7.48 | 0.08 | 7.39 | 0.08 | 297 | 0.37 | ns |
| S/I KO% | 19.7% | 0.2% | 19.7% | 0.2% | 19.5% | 0.2% | 297 | 0.36 | ns |
| Hot carcass (kg) | 19.93 | 0.09 | 19.95 | 0.09 | 19.99 | 0.09 | 297 | 0.13 | ns |
| Hot carcass KO% | 52.6% | 0.2% | 52.7% | 0.2% | 52.8% | 0.2% | 297 | 0.19 | ns |
| Processing Time | 00:51 | 00:01 | 00:51 | 00:01 | 00:53 | 00:01 | 297 | 1.78 | ns |
| Heads & feet (kg) | 3.63 | 0.03 | 3.67 | 0.03 | 3.64 | 0.03 | 297 | 0.40 | ns |
| Skin (kg) | 3.11 | 0.04 | 3.13 | 0.04 | 3.21 | 0.04 | 297 | 2.07 | ns |
| Liver (kg) | 0.749 | 0.008 | 0.731 | 0.008 | 0.744 | 0.008 | 297 | 1.32 | ns |
| Lungs (kg) | 0.463 ^b | 0.007 | 0.418 ^a | 0.007 | 0.426 ^a | 0.007 | 297 | 12.64 | *** |
| Heart (kg) | 0.161 | 0.002 | 0.162 | 0.002 | 0.161 | 0.002 | 297 | 0.13 | ns |
| Spleen (kg) | 0.067 | 0.001 | 0.066 | 0.001 | 0.068 | 0.001 | 297 | 0.70 | ns |
| Gall bladder (kg) | 0.026 | 0.001 | 0.027 | 0.001 | 0.028 | 0.001 | 297 | 0.46 | ns |
| Pluck Remainder (kg) | 0.206 ^b | 0.003 | 0.191 ^a | 0.003 | 0.203 ^b | 0.003 | 297 | 6.35 | ** |
| UA weight (kg) | 0.202 | 0.021 | 0.192 | 0.021 | 0.221 | 0.021 | 297 | 0.51 | ns |

Values in a row with different superscripts differ significantly.

df = degrees of freedom. F = variance ratio

ns = not significant. Significance at * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Significance levels separated by a comma indicate respective levels of significance in the order of the columns TRS, EHOS, PCEHOS.

S/I = Stomach/Intestine, KO% (Kill-Out %) = percentage of that animal product over the live-weight, Processing Time = the time (in hours and minutes) between the last live-weight and the final carcass weight, Pluck Remainder = the remainder of the red offal or 'pluck', UA = Unaccounted Weight after deducting 4 min blood, carcass and all by-products from the live-weight.

of EHOS were the same between publications whilst the TRS profiles were different. In this experiment the TRS rate of blood loss was found to be more constant whilst Anil et al. (2004) showed that the TRS rate closely followed the EHOS profile. This could be because the effect of gravity on a vertically hanging carcass in the Anil et al. paper may have a greater effect than a beating heart alone.

Since the animals were slaughtered and held upright for 120 s, the sticking wound was located higher than the heart. 70% of the final blood loss was lost within that 120 s period. Research has demonstrated that a beating heart is unlikely to directly affect the drainage of blood from the carcass (Warriss, 1984). Kirton et al. (1981) concluded that cardiac arrest significantly reduced the amount of blood lost at slaughter but the 'missing' blood was not retained in the meat and was most likely lost during carcass dressing or retained in the viscera. If the heart is to remain viable as a pump, venous blood pressure must be maintained in order for the heart to refill during diastole. Severance of major blood vessels in the neck will result in a catastrophic fall in blood pressure with a resultant fall in cardiac output. Kirton et al. (1978) found that the time from sticking to zero pressure measured in the femoral artery of EHOS lambs stunned with 100–200 V for 2 s, ranged from 27–100 s (mean 47 s) but it is unlikely that venous pressure is maintained for such a length of time. Although the heart may continue to beat for more than 10 min after slaughter (Newhook & Blackmore, 1982) the heart's function as a pump ceases once the venous pressure drops below a critical limit however, little research has been conducted to establish the pressure or time at which this occurs. The primary driver of blood loss is likely to be gravity aided by muscle pumping

produced by either post-stun convulsions or, convulsions produced by spinal reflexes, post-brain death (personal observations).

For all treatments there appeared to be an inverse relationship between S/I and 4-minute blood loss. This suggests that some of the weight accounted for as blood lost may in fact have been rumen discharge. The lowest S/I weight (for TRS) correlated with the highest 4-minute blood loss and vice-versa. The higher UA in TRS could be due to increased stomach content losses since the average TRS S/I weight was lighter than the others. The extra processing time for the Experimental protocol (approximately 50% longer) did not lead to large differences in UA.

4.2. Commercial protocol

The perceived lower blood loss of PCEHOS highlighted in Group 3 could be due to reduced rumen discharge because of a lower initial S/I weight and higher blood retention on the fleece. The skin is known to harbour residual blood so the extra weight of Group 3 PCEHOS skin could be due to higher blood retention compared to others.

Higher lung weights in TRS for Group 4 and 5 could be due to a higher likelihood of blood flowing down the trachea in an upright position (Gregory, von Wenzlawowicz, & von Holleben, 2009). However this was not found in Groups 1,2 and 3 and Gregory, Shaw, Whitford, and Patterson-Kane (2006) found that the prevalence of carotid ballooning (>1 cm diameter) to be the least in sheep (0.1% incidence) when compared with other species.

Table 5

Subjective categorisation of stun voltage profiles.

| Profile type | Good | Wave modulated | Unusual | Total |
|---------------------|------|----------------|---------|-------|
| Experimental EHOS | 0 | 22 | 1 | 23 |
| Experimental PCEHOS | 4 | 12 | 4 | 20 |
| Commercial EHOS | 19 | 15 | 0 | 34 |
| Commercial PCEHOS | 23 | 9 | 1 | 33 |

Table 6

Experimental and commercial voltage RMS data analyses.

| Treatment | EHOS | | | PCEHOS | | | df | F | Sig |
|--------------------------|------|------|----|--------|------|----|----|------|-----|
| | N | Mean | SE | N | Mean | SE | | | |
| Experimental voltage RMS | 23 | 226 | 8 | 20 | 223 | 8 | 41 | 0.08 | ns |
| Commercial voltage RMS | 34 | 221 | 5 | 33 | 218 | 5 | 65 | 0.16 | ns |

4.3. General discussion

The 4-minute blood loss % values are similar between the Experimental and Commercial protocols. Kirton et al. (1981) found no difference in blood lost at exsanguination between TRS lambs (with the spinal cord cut) and EHOS lambs after 120 s. Warriss (1984) stated that sheep lose approximately 4% of their live-weight in blood at exsanguination whereas, in contrast, this research found greater blood loss percentages ranging from 4.7% to 5.2%. This is likely due to interference by rumen content loss since lambs were allowed access to feed and water ad-lib overnight. An approximation of the rumen content loss can be derived by subtracting Experimental 120 s BL% from Commercial 2 minute BL%. This provides potential rumen content data of 1.0%, 1.1% and 1.0% for TRS, EHOS and PCEHOS respectively but this is an upper limit as it would also include further blood loss due to the change in orientation and gravity effect of vertical hanging. However as each batch had access to the same feed resources it is the difference between the treatments that is of scientific interest.

Warriss and Leach (1978) showed that the slaughter position affects bleeding and, in agreement, this research found blood loss significantly increased when carcasses were hung vertically on the line after slaughter rather than kept upright in the V-restraint for the initial 2 minute period. Velarde et al. (2003) found that EHOS lambs had a significantly higher BL% than TRS lambs after 1 min. They used 43 light lambs (approximately 19–21 kg live-weight) and found bleed out percentages of approximately 4.2–4.7%. This percentage range was similar for the Commercial protocol at 1 min but was significantly larger than the Experimental protocol. This is most likely due to change in animal orientation and gravity as Velarde et al. (2003) hung all animals vertically on the bleed-rail before bleeding took place. Furthermore it is likely that the smaller blood collection time of 1 min was insufficient to allow for the initially slower rate of blood loss seen with Experimental TRS animals compared to the faster rate seen with electrically stunned animals.

Comparing blood loss data at 2 min between the Experimental and Commercial protocols suggest that carcasses hung vertically lose approximately 0.4 kg more 'blood' than those restrained upright in a V-restraint during this period. Thus, in agreement with Velarde et al. (2014), a change in carcass orientation also aids blood lost at exsanguination. This figure should be treated cautiously as an 'upper range' estimate since it will undoubtedly include some rumen release. Warriss (1984) concluded that any blood not lost at sticking is probably retained in the viscera rather than the carcass. The KO% for liver, lungs and heart were in agreement with Hansard (1956).

4.4. Future research priorities

The time, following a full deep ventral neck cut, at which the venous pressure drops below critical levels thereby disabling the heart from functioning as a pump should be investigated. Blackmore and Newhook (1976) suggested that sheep bled in the lateral recumbency position lose approximately 10% more blood than those hung vertically. It would be useful to repeat this experiment to include the effect of lateral recumbency, taking welfare optimization into account. Warriss and Leach (1978) found that there is no correlation between blood lost on exsanguination and blood retained in the muscle but updated research in this field to include PCEHOS as a treatment should be conducted.

5. Conclusion

Despite the results of Group 3, overall there was no significant difference in blood lost at exsanguination between TRS, EHOS and PCEHOS for either Experimental or Commercial protocols. The rate of blood loss was quickest in EHOS and PCEHOS followed by TRS. The final blood loss values included some inevitable rumen contamination but, given that all lambs had access to the same feed resources and slaughter treatments were randomised, there was no difference between treatments. A post-

slaughter change in carcass orientation from an upright position to vertical hanging aids blood lost at exsanguination. The bleeding of sheep at exsanguination lasts for a minimum of 2 min after which the amount of blood released is negligible. Although it may carry on beating for many minutes the heart's function as a pump ceases once the venous pressure drops below critical levels but little research has been conducted to investigate the time or pressure at which this occurs. This research is anticipated to inform discussion on the merits of different slaughter methods compatible with Halal requirements.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.meatsci.2015.06.008>.

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