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Evaluation of colour and bleeding efficiency of imperfectly bled, scientifically slaughtered and cold slaughtered beef carcasses#

Vandana Sasidharan[1*], V. N. Vasudevan[2], B. Sunil[3], T. Sathu[2] , V. Ramnath[4] , K. J. Arun Sankar[1] and R . Yazhinidevi[1]
Department of Livestock Products Technology,
College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680 651.
Kerala Veterinary and Animal Sciences University, Kerala, India.

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Abstract

The present study was carried out at the Meat Technology Unit, Kerala Veterinary and Animal Sciences University, Mannuthy to examine the colour and bleeding efficiency of beef carcasses subjected to varying levels of bleeding. Muscle samples were collected from imperfectly bled (IB), scientifically slaughtered (SS) and cold slaughtered (CS) beef carcasses and were packed in HDPE packages. The Hunter L a* b* values were analysed on the 0,2,4 and 6 days of refrigerated storage at 4±1°C and the bleeding efficiency was studied on the day of slaughter. From the results it was concluded that malachite green test (MGT) could be assigned as a test to detect complete bleeding. However only lower proportions of IB and CS samples showed positive results on MGT assay, which makes it a less reliable method for detecting the efficiency of bleeding. The mean haemoglobin (Hb) concentration (mg/g) of IB and CS carcasses (0.07±0.003 and 0.09±0.008, respectively) were significantly ($p<0.01$) higher than that from SS carcasses (0.05±0.004). There was no significant difference between IB and SS groups for the blood loss parameters considered. The mean number of turgid intercostal veins for IB (7.83±0.98), SS carcasses (6.5±0.88) was significantly ($p<0.01$) lower to that of CS carcasses (10.5±0.43). More than 10 turgid intercostal veins in a bovine carcass may be indicative of cold slaughter and absence of bleeding. No significant difference was noted between groups on all days for a* value and on all days except day 2 for L* and b* values.*

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1. MVSc Scholar,

2. Assistant Professor,

3. Professor and Head,

4. Professor and Head, Department of Veterinary Physiology

**Corresponding author: vandana.sasidharan474@gmail.com, Ph: 8593084742*

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Meat is a valuable commodity and an important source of protein. Meat adulteration is one of the major global concerns with the situation getting worse in developing countries like India. One of the illegal activities in this industry is the sale of meat of dead animals, commonly known as the cold slaughtered meat. Since the cold slaughtered meat is from dead animal and not from a healthy live animal, it does not undergo one of the vital steps of meat processing, which is sticking/bleeding/ exsanguination and has a major significance form the point of view of public health as well as religious sentiments.

Bleeding efficiency is an essential requirement of slaughter procedures in order to obtain a high-quality product. Blood loss is a major concern for meat processors as the residual blood in the carcass is often related to a decrease in shelf life and meaty flavour. The high nutritive value, favourable pH, temperature, relative humidity and water activity makes blood an ideal medium for microbial proliferation (Addeen *et al.*, 2014). Thus, optimising blood loss at slaughter to ensure product quality, promote shelf and reduce meat and carcass defects is a major concern of the meat processing industry (Ali *et al.*, 2011). Meat colour is determined by the concentration of pigments, the reactions of the pigments with gaseous elements or compounds and the structural properties of muscle proteins. The concentration of haemoglobin (Hb) is the main determiner for meat colour development and slaughter method is one of the contributory factors for Hb content remaining in meat (Ahmad *et al*., 2015). The present study investigated the effect of imperfect bleeding representing controlled bleeding, scientific slaughter representing complete bleeding and cold slaughter representing no bleeding on the mean Hb content, blood loss and colour of muscle samples. The efficiency of malachite green test (MGT), one of the popular dye tests for detecting bleeding efficiency was also done in detecting its effectiveness as a method in detecting residual blood.

Materials and methods

Muscle sampling

Twelve female cross bred cattle in the age group of 4 to 6 years from various farms under the Kerala Veterinary and Animal Sciences University were utilised in this study. Six animals were subjected to imperfect bleeding wherein bleeding was arrested by clamping either of the carotid arteries post-stunning using an artery forceps for a period of 120 s followed by bilateral severance of carotid artery and jugular vein by throat incision. The other group of six animals were slaughtered as per scientific procedures including mechanical stunning followed by bilateral severance of the carotid arteries for complete bleeding. The bleeding period continued for six minutes.

Muscle samples from six female cross bred cattle in the age group of 4 to 6 years and dead due to natural causes and presented at the Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy for post-mortem examination were used for the collection of cold slaughtered meat.

Muscle samples representing four wholesale cuts namely; chuck, rib, loin and round were immediately harvested, portioned and packed in HDPE pouches and stored in a domestic refrigerator maintained at 4±1°C for the estimation of colour at 0, 2, 4 and 6 days. The various blood loss parameters were also analysed on the day of slaughter.

Hunetr L a* b* values*

Hunter L* a* b* values of imperfectly bled (IB), scientifically slaughtered (SS) and cold slaughtered (CS) muscle samples were objectively determined on the 0, 2, 4 and 6 days using Mini Scan XE plus Spectrophotometer (Hunter Lab, Virginia, USA) with diffuse illumination.

Estimation of bleeding efficiency

The extent of blood removal was qualitatively assessed by MGT following the procedure described by Kowale *et al*. (2008). Haeme proteins from the muscle samples

of IB, SS and CS animals were extracted following the method of O'Brien *et al.* (1992). The concentration of Hb was evaluated using a modified kinetic technique of Goyal and Basak (2009). The various blood loss variables were calculated following a modification of procedure described by Anil *et al.* (2006). The intercostal veins of differently bled animals were subjectively evaluated for the presence of blood as a function of efficiency of bleeding. The intercostal veins were examined and the number of veins filled with more than 50 per cent of their length with blood were categorised as turgid and their number was recorded.

Data analysis

The data recorded were analysed statistically as per Snedecor and Cochran (1994) by repeated ANOVA measures, chi square test for multiple proportions and one way ANOVA by using SPSS Software Version 24.0.

Results and discussion

Effect of slaughter method on Hunter L* value

The mean Hunter L* a* b* values from day 0 to day 6 of refrigerated storages of muscle samples from the three animal groups are depicted in Table 1. Lightness or darkness of meat is denoted by Hunter L* values. The variations in L* values have been attributed to the changes that takes place in the structure of meat during ageing, especially protein denaturation, which results in greater dispersion and, thus, increased lightness (MacDougall, 1982). The mean L* values of IB, SS and CS samples on the fresh product stage was found to be 25.61 ± 2.27, 29.90 ± 2.39 and 23.07 ± 0.99 respectively. The L* values were found numerically lowest for CS samples, indicating a darker sample and highest for SS samples, indicating a lighter samples on the day of slaughter and sampling. The lightness increased significantly ($p<0.01$) to 33.61 ± 0.78 and 30.61 ± 0.69 on the second day for IB and CS samples respectively. Insausti *et al.* (2000) reported that the L* value increased progressively during storage of beef. Refrigerated storage had no effect on the L* value of SS samples, although a non-significant increase was there from day zero to day six. On the final day of storage study, the L* value was lowest for CS samples (31.38 ± 1.19) and highest for SS samples (35.32 ± 0.32). Sohaib *et al*. (2020) also reported a higher L* value for halal slaughtered bird meat compared to meat from dead and decapitated birds.

Table 1. Mean L* a* b* values of imperfectly bled (IB), scientifically slaughtered (SS) and cold slaughtered (CS) meat samples during storage (Mean±S.E.)

Colour	Storage days (days)			
	0	2	4	6
L* value				
IB	25.61 ± 2.27^{b}	33.61 ± 0.78^{Aa}	31.98 ± 1.77^{a}	34.25 ± 2.37^{a}
SS	29.90 ± 2.39	30.50 ± 0.68^{B}	33.89 ± 0.81	35.32 ± 0.32
CS	23.07 ± 0.99^{b}	30.61 ± 0.69^{aB}	31.47 ± 0.81^{a}	31.38 ± 1.19^{a}
a* value				
IB	9.87 ± 0.72^{b}	13.09 ± 0.57^{a}	12.15 ± 0.46^{ab}	11.93 ± 0.9^{ab}
SS	11.13 ± 0.33^{b}	12.96 ± 0.57^{a}	12.35 ± 0.19^{a}	10.64 ± 0.12^{b}
CS	9.62 ± 0.20^{b}	12.09 ± 0.34^{a}	13.6 ± 1.04^{a}	12.44 ± 0.45^{a}
b* value				
IB	6.84 ± 0.81^{c}	11.65 ± 0.4^{aA}	10.11 ± 0.45^{b}	11.4 ± 0.66^{a}
SS	8.1 ± 1.12	9.93 ± 0.36^{B}	10.97 ± 0.15	10.56 ± 0.15
CS	6.82 ± 0.43^{c}	10.23 ± 0.27^{bB}	11.62 ± 0.57^{a}	10.61 ± 0.57^{ab}

** Significant at 0.01 level; ns non-significant

Means having different small letter as superscript differ significantly within a row

Means having different capital letter as superscript differ significantly within a column

Effect of slaughter method on Hunter a* value

Hunter a* value indicates the redness or greenness of the meat. Acceptability of consumer is influenced by the redness of the meat. There was no significant difference between in a* values of the studied animal groups on all days of storage. The a* value significantly ($p<0.01$) increased from 9.87 ± 0.72 on day 0 to 13.09 ± 0.57 on day 6 in case of IB samples, from 11.13 ± 0.33 on day 0 to 12.96 ± 0.57 on day 6 in case of SS samples and from 9.62 ± 0.20 to 12.09 ± 0.34 in case of CS samples indicating an improvement in redness during the course of storage. The SS samples were found to be redder on the day of sampling while it was numerically higher for IB samples (13.09 ± 0.57) on the second day of the study. Myoglobin is the primary protein pigment associated with meat color. Most of the muscle Hb is lost during exsanguination and has been reported to contribute between 6-16 per cent of total fresh meat pigments depending on muscle and the method used to quantify Hb (Crystal *et al*., 1981), thus Hb has lesser influence on meat colour compared to Mb.

Effect of slaughter method on Hunter b* value

With respect to the b* value, no significant difference was observed between treatments on all days of the study except on day 2. On the 2nd day, the b* value of IB samples were found to be significantly higher ($p<0.01$) to CS and SS samples. The b* values increased from 6.84 ± 0.81, 8.1 ± 1.12, 6.82 ± 0.43 at day zero to 11.4 ± 0.66, 10.56 ± 0.15 and 10.61 ± 0.57 for IB, CS and SS samples respectively. Hunter b* values indicates the yellowness or blueness of the muscle with positive value indicating yellowness. Insausti *et al.* (2000) reported that during refrigerated storage of beef yellowness (b*) increased during the first seven days and then decreased slightly. Onenc and Kaya (2004) reported a steady b* value during storage up to nine days.

Malachite Green Test

Proportion of samples from IB, SS and CS carcasses showing imperfect bleeding in malachite green test (MGT) is given in Table 2. Chi square test for multiple proportions was done for comparing the percentage of similar proportions under different groups. As the chi-square value was found to be significant z-test for two independent proportions was done for pair wise comparison. The MGT is a conventional test for the qualitative determination of the efficiency of bleeding. Cloudy green colour in reaction mixture is indicative of imperfect bleeding and clear blue reaction mixture is indicative of perfect bleeding as per literature. In our study, MGT was able to detect 33.33 per cent of the samples from IB category and 25 per cent of the samples from CS category to be imperfectly bled. A 100 per cent of SS samples showed perfect bleeding. From the results it can be concluded that MGT could be assigned as a test to detect complete bleeding. However only lower proportions of IB and CS samples showed positive results on MGT assay, which makes it a less reliable method for detecting the efficiency of bleeding. The MGT is a semi quantitative test and it detected only three grades of bleeding, namely, normal, incomplete and unsatisfactory (Warris, 1977). Moreover, both Mb and Hb from the meat extract participated in the reaction and hence the test may exclusively determine residual blood levels in muscle samples. Hence differences in the Mb levels in the samples might have contributed to varying positive reaction to MGT in IB and CS samples.

Haemoglobin estimation

The mean haemoglobin (Hb) concentrations of muscle samples from IB, SS and CS carcasses are presented in Table 3. The mean Hb concentration (mg/g) of IB and CS carcasses (0.07±0.003 and 0.09±0.008, respectively) were significantly ($p<0.01$) higher than that from SS carcasses (0.05±0.004). The meat Hb content depended on the extent of carcass bleeding and the vascular bed in the muscles (Oellingrath *et al*., 1990). Thus, carcasses subjected to no or minimal bleeding retained more blood in muscles which was reflected in the mean Hb content. Alvarado *et al*. (2007) reported a Hb content in normally bled birds was about 13 to 17 per cent lower when compared with not bled birds. They also reported a higher Hb for unbled samples compared to bled group.

Table 2. Proportion of samples from IB, SS and CS carcasses showing imperfect bleeding in malachite green test

Animal group	Number of samples Analysed	Number of samples showing imperfect bleeding in MGT	
		Number	Per cent
IB	24	8 out of 24	33.3[b]
SS	24	0 out of 24	0[a]
CS	24	6 out of 24	25.0[b]

** Significant at 0.01 level. Percentage having different letter as superscript differ significantly.

Table 3. Mean haemoglobin (Hb) content (mg/g) of imperfectly bled (IB), scientifically slaughtered (SS) and cold slaughtered (CS) carcasses and the various primal cuts (Mean±S.E.)

Animal group	IB	SS	CS
Mean Hb concentration (mg/g)	0.07±0.003[A]	0.05±0.004[B]	0.09±0.008[A]
Mean number of turgid intercostal veins	7.83±0.98[A]	6.5±0.88[A]	10.5±0.43[B]

** Significant at 0.01 level; ns non-significant
Means having different upper case alphabets as superscript differ significantly within a row

Gross appearance of intercostal veins

The mean number of turgid intercostal veins for IB, SS and CS carcasses is depicted in Table 3. The mean number of intercostal veins showing turgidity with blood were 7.83± 0.98, 6.5 ± 0.88 and 105 ± 0.43, respectively. The number of intercostal veins which showed turgidity ranged from 4 to 10 in case of IB and SS carcasses and from 9 to 12 in case of CS carcasses. The mean number of turgid intercostal veins was significantly ($p<0.01$) higher in CS carcasses than that in IB and SS carcasses. The results indicate that more than 10 turgid intercostal veins in a bovine carcass may be indicative of cold slaughter and absence of bleeding. Examination of more number of cold slaughtered bovine carcasses is warranted to arrive at a definitive conclusion.

Determination of blood loss

The various blood loss parameters evaluated are given in Table 4. There was no significant difference between the two variably bled animal groups (IB and SS groups; no bleeding in CS animal group) for the parameters considered. The mean quantity of blood collected during slaughter of imperfectly bled animals was 11.38±0.72 kg and that during scientific slaughter procedure was 10.22± 0.48 kg. Vimini *et al*. (1983) reported that the weight of blood removed from animals decreased as the time between stunning and bleeding increased. These authors suggested that muscle contraction and gravity and no heart activity, were the major factors in removing blood from the musculature of slaughter animals.

Table 4. The various blood loss variables of imperfectly bled (IB) and scientifically slaughtered (SS) animals (Mean±S.E)

Blood loss Variable	IB	SS	F Value (p value)
Total blood loss (kg)	11.38±0.72	10.22± 0.48	0.522(0.487)[ns]
Live weight(kg)	306 ± 24.52	257 ± 35.53	1.245(0.291)[ns]
Estimated total blood weight(kg)	18.34±1.47	15.46±2.13	0.029(0.868)[ns]
Estimated % blood loss	63.35±4.89	69.61±5.86	0.094(0.766)[ns]
Blood loss as a % of live wt	3.79±0.29	4.17±0.35	0.094(0.766)[ns]

ns - Non-significant

The animals subjected to imperfect bleeding lost an estimated 63.35 ± 4.89 per cent of their blood, compared to the 69.61 ± 4.89 per cent estimated blood loss for scientifically slaughtered animal, which were not significantly different. The IB animals lost 3.79 ± 0.29 per cent of their live weight compared to a numerically higher percentage (4.17 ± 0.35 per cent) among SS group animals. Wadhwani *et al*. (2011) concluded that the measurements of slaughter blood cannot be used as a reliable index of blood remaining in the meat since this quantity does not include the blood remaining in the viscera, skin and other inedible parts of the body, particularly the heart, liver and lungs, which retain large volumes of blood.

Conclusion

The present study concluded that minimal or no bleeding results in greater amount of Hb to be retained in the muscles. Moreover, blood loss during slaughter cannot be used as a reliable method to indicate the extent of bleeding as some of the blood may remain in the viscera as well. More than 10 turgid intercostal veins may be indicative of absence of bleeding and may be practiced during routine post-mortem examinations to ascertain efficiency of bleeding. Malachite green test could be assigned as a test to detect complete bleeding. However only lower proportions of IB and CS samples showed positive results on MGT assay, which makes it a less reliable method for detecting the efficiency of bleeding. The L* value was higher for CS samples indicating a darker carcass. The a* value was higher for SS samples, indicating that Mb has more influence on the development of colour compared to Hb level. Studies with greater number of carcasses and carcasses from other species can be a subject of research in future studies.

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Conflict of interest

The authors declare that they have no conflict of interest

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