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Anandhu A Kumar
Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala, India

Vasudevan VN
Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala, India

Sathu T
Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala, India

Sreekumar TR
Department of Veterinary Physiology, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala, India

Irshad A
Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala, India

Anoop S
Department of Veterinary Surgery and Radiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

Corresponding Author:
Anandhu A Kumar
Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala, India

Analysis of total and heme iron contents in *Biceps femoris* from rabbits subjected to two mechanical stunning techniques

Anandhu A Kumar, Vasudevan VN, Sathu T, Sreekumar TR, Irshad A and Anoop S

Abstract
This study is aimed to assess the total iron (TFe) and heme iron (HeFe)contents in meat obtained from rabbits which were subjected stunning by a developed stunner (DS, n=12) and by the percussive blow method (PB, n=12). Female crossbred (Soviet Chinchilla x White Giant) rabbits weighing 1.5-2 kg were selected as subjects for this investigation. Following stunning and scientific slaughtering procedures, samples of *Biceps femor*is muscle were harvested. The quantification of TFe concentration in these samples involved an acid digestion process, followed by atomic absorption spectrophotometry (AAS). The HeFe content of *Biceps femor*is muscle was determined through the subtraction of non-heme iron (nHeFe) content from the total iron. Initially, the nHeFe content was assessed using an acid extraction technique along with AAS. The study results revealed that the mean TFe concentrations (mg/100 g) in meat from rabbits of DS groups were 0.168±0.008 and 0.102±0.013 and those from PB group were 0.185±0.009 and 0.182±0.009, respectively. The TFe content consistently remained below 1 mg/100 g in all meat cuts. Furthermore, approximately 60 percent of the iron content in mechanically stunned rabbit meat was found to be in the form of heme iron. The heme iron content in *Biceps femor*is was observed to be higher in the traditional percussive blow group compared to the developed stunner group. These findings indicate that *Biceps femor*is region of rabbit possesses low TFe concentrations, similarly to poultry, with the majority of iron existing in the form of heme iron. The stunning process with developed stunner induced more efficient bleeding in rabbits compared to the traditional percussive blow method.

Keywords: Rabbit meat, stunning, percussive blow, heme iron, atomic absorption spectrophotometry, acid extraction technique

Introduction
Iron is an essential nutrient for humans, playing a vital role in various physiological processes, including oxygen transport, DNA synthesis, and energy production (Cairo *et al*., 2006) [1]. A significant portion of dietary iron intake comes from meat consumption, making it imperative to understand the iron content and its bioavailability in different meat sources (Lynch *et al*., 1989) [6]. Among these sources, rabbit meat has gained recognition for its nutritional value, being lean, high in protein, and relatively low in fat. In the context of iron, two primary forms were of particular interest: total iron (TFe) and heme iron (HeFe). TFe represents the overall iron content in meat, while HeFe is the iron bound within heme molecules, predominantly found in animal tissues. HeFe is known for its high bioavailability compared to non-heme iron, making it a crucial consideration for dietary iron intake (Geissler *et al*., 2011) [3]. The method of stunning animals before slaughter has evolved over time, with traditional percussive blows being replaced by more humane and efficient methods such as penetrative stunners. However, the impact of stunning methods on the iron composition of meat remains a relatively unexplored area of research. Understanding the differences in iron content between rabbit stunned with a developed stunner and that stunned using the traditional percussive blow could provide valuable insights for both the meat industry and consumers.
This study seeks to address this knowledge gap by conducting a comparative analysis of the total iron and heme iron content in rabbit meat processed through these two distinct stunning methods. The results of this investigation may have implications not only for the nutritional quality of rabbit meat but also for animal welfare considerations within the meat production industry. By shedding light on the potential variations in iron content arising from different stunning techniques, this research contributes to a more comprehensive understanding of the

nutritional profile of rabbit meat and the broader implications for consumers and the industry alike.

Materials and Methods

The research procedure received formal approval from the Institutional Animal Ethics Committee (IAEC) at the College of Veterinary and Animal Sciences in Mannuthy. Female crossbred (Soviet Chinchilla x White Giant) weighing 1.5 – 2 kg were procured from a local farmer and brought to the MTU for the purpose of slaughter and meat production. All the animals were in the age group of five months maintenance on a uniform concentrate diet with green fodder supplementation. These rabbits were included in the current study for the comparative evaluation of heme iron content in meat samples between two stunning methods: Developed stunner (DS, n=12) (Fig 1) and the traditional percussive blow technique (PB, n=12).

Following the stunning procedure and subsequent steps of sticking and deskinning, samples weighing approximately 20 g each of *Biceps femor*is muscle were collected from animals of both the groups. Each individual sample was placed inside a sealed polyethylene bag and marked with the respective animal's identification number. These samples were then preserved at a temperature of -18 °C until the point of analysis. Prior to determining the TFe content, the samples were thawed, and any excess fat was carefully removed to ensure that the analysis focused exclusively on the lean muscle mass.

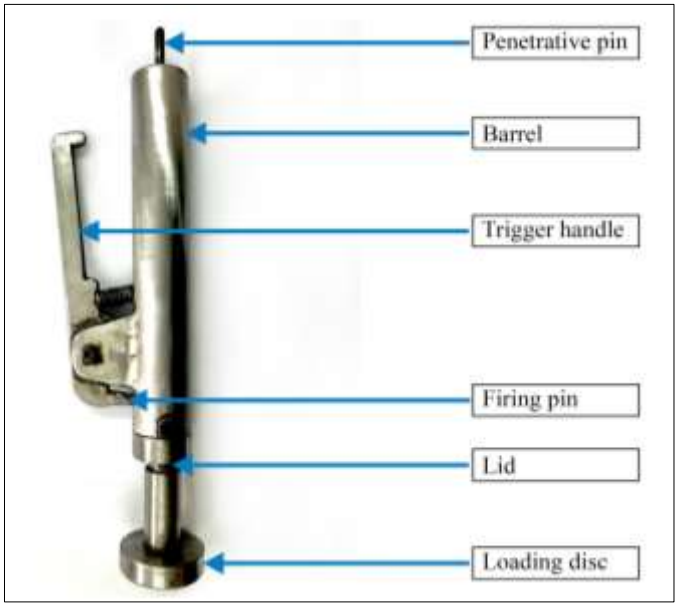

Fig 1: Developed Stunner

Fig 2: Stunning with Developed Stunner

Determination of TFe and HeFe

The heme iron content in the muscle samples from both the groups was determined as per the procedure by Valenzuela *et al*. (2009) [9]. *Biceps femor*is was initially subjected to digestion in concentrated nitric acid using a microwave digester (PerkinElmer, Titan MPS) (combining 0.5 g of meat sample with 7 ml of 70% concentrated nitric acid). The mixture was gently agitated, and the vessel was sealed after allowing it to sit for a minimum of 10 minutes. Subsequently, the mixture underwent heating in the microwave digester according to the following program.

Step	Target Temp (ºC)	Pressure Max (bar)	Ramp Time (min)	Hold Time (min)	Power (%)
1	170	30	10	5	80
2	200	30	1	20	90
3	50	30	1	10	0

Subsequently, the digested samples were analyzed utilizing a Flame Atomic Absorption Spectrophotometer (AAS) (Perkin Elmer, PinnAAcle 900H model, 248.33 nm). Calibration blank solution used throughout was 2% w/v HNO_3.

The non-heme iron (nHeFe) content was quantified through an acid extraction technique as described by Rebouche *et al*. (2004) [8]. *Biceps femor*is from rabbit meat tissue homogenates were prepared in the ratio of 1:10 (w/v) in high-purity water, using mortar and pestle. Equal volumes (20 µl of each) of tissue homogenates and protein precipitation solution (1 N HCl and 10% trichloroacetic acid in high-purity water) were combined in boil proof 0.5 ml eppendorf tubes, vortex mixed, and placed in a 95 °C hot air oven (ROTEK, B&C Industries) for 1 h. Tubes and contents were cooled in water at room temperature for 2 min, vortex mixed, and then centrifuged at 8200 g for 10 min. Supernatant aliquots (30 µl) were mixed with 30 µl of chromogen solution (0.508 mmol/l

ferrozine, 1.5 mol/l sodium acetate and 0.1% or 1.5% (v/v) thioglycolic acid in high purity water). Sample blanks consisted of 30 µl of tissue extract supernatant and 30 µl of 1.5 mol/l sodium acetate containing 0.1% or 1.5% (v/v) thioglycolic acid. After 30 min at room temperature, absorbance was measured using a UV-VIS spectrophotometer (PerkinElmer, Lambda 750) at 562 nm. Standard curves for non-heme iron content estimation (Fig 6) were prepared using iron standards containing 0.5, 1, 2, 4 and 8 µg/ml of the iron standard solution and diluted with an equal volume of protein precipitation solution. Subsequently, the HeFe content was calculated as the difference between the Total Iron (TFe) and the non-heme iron (nHeFe) content, thereby providing an accurate determination of the heme iron concentration within the samples.

Statistical Analysis

Descriptive statistics were computed for all variables. An independent t-test was employed to assess any statistically significant differences in the levels of iron and heme iron contents in the muscle samples.

Results and Discussion

Table 1 displays the mean values for TFe, HeFe, and non-HeFe content (mg/100 g) in meat from the animals of DS and PB groups. All the meat samples contained TFe concentrations below 1 mg/100 g, with average values of 0.168±0.008 for DS group and 0.185±0.009 for PB group. HeFe was primarily located within haemoglobin and myoglobin molecules, where it plays a vital role in facilitating tissue oxygenation, as noted by Gidding (1977) [4]. This type of iron was more easily absorbed by the body, with absorption rates ranging from 10% to 30%, in contrast to non-heme iron, which has absorption rates between 1% and 20%, as indicated by Hallberg (1981) [5].

Table 1: Mean total, heme and non-heme iron contents in meat samples from rabbits stunned with the penetrative stunner and the percussive blow method (mg/100 g)

Variables (mg/100 g)	Animal Group		Z-value	P-value
	DS (n=12)	PB (n=12)		
Total Iron	0.168±0.008	0.185±0.009	0.76^{ns}	0.472
Heme Iron	0.102±0.013	0.182±0.009	3.05*	0.019
Non-heme iron	0.065±0.012	0.004±0.001	3.20*	0.024

* Significant at 0.05 level (p<0.05); ns-not significant
DS-developed stunner PB-Percussive blow

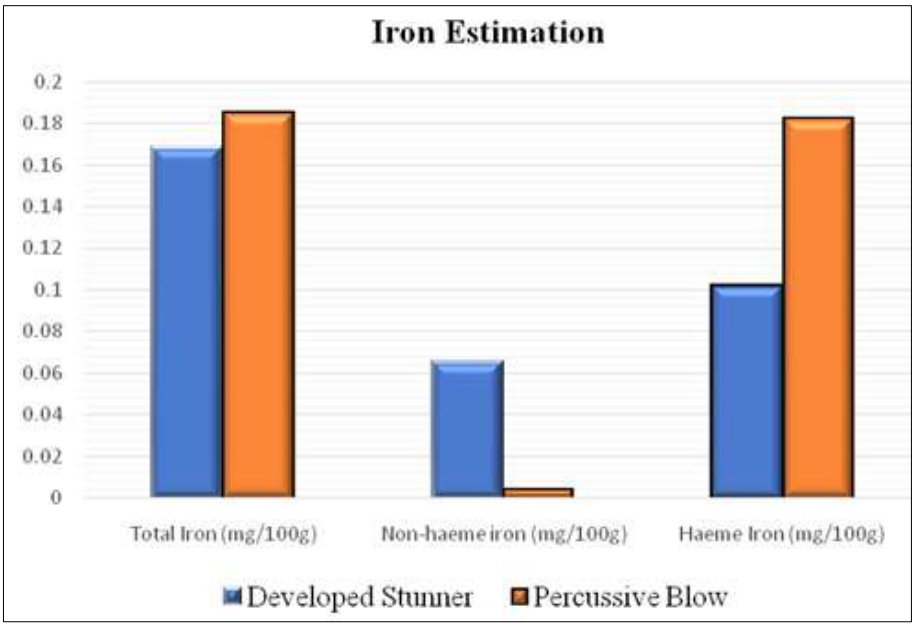

Fig 4: Mean total, heme and non-heme iron contents in meat samples from rabbits stunned with the developed stunner and the percussive blow method (mg/100 g)

Martínez-Torres *et al*. (1986) [7], documented that HeFe constitutes 55–65% of the total iron (TFe) in beef meat [7]. Similarly, Valenzuela *et al*. (2009) [9] found that approximately 65% of TFe in bovine meat was comprised of HeFe. Additionally, when examining *Biceps femor*is from rabbit meat obtained through DS, it was noted that the HeFe content was approximately 60% of the total iron.

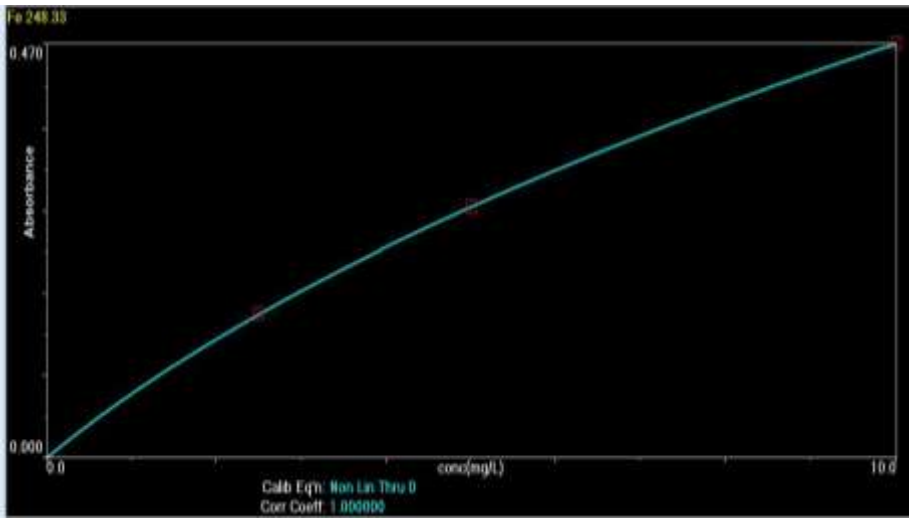

Fig 5: Calibration curve for iron standards for iron estimation

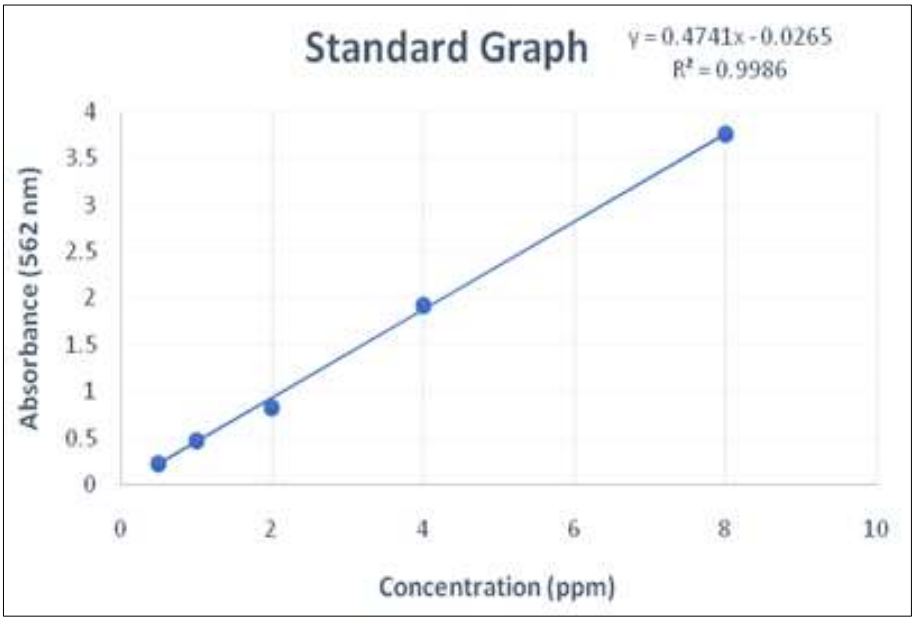

Fig 6: Calibration curve for iron standards for non-heme iron estimation

The when stunning using developed stunner was employed, there was a slightly lower total iron content compared to the percussive blow methods. This suggests that the selection of the stunning method did not exert a substantial influence on the total iron content observed in the samples.

Dalle Zotte *et al*. (1998) [2] noted that the efficiency of blood removal process, known as exsanguination, could be a crucial factor in determining the iron content, as any residual blood remaining in the muscle tissue could impact the iron content of the muscle [2]. The heme iron contents in meat samples from animals of DS and PB groups were 0.102±0.013 and 0.182±0.009, respectively. Valenzuela *et al*. (2009) [9] reported rabbit meat had a heme iron content of 0.57±0.09 (indicating that 69 percent of the sample is heme iron) [9]. The heme iron content in samples from DS group (approximately 60 percent of TFe) was significantly lower than he PB group. Assuming a constant myoglobin content in the identical muscles of rabbits reared under similar conditions, the lower proportion of HeFe suggests that muscle tissue from animals subjected to DS group retains less blood, potentially indicating a more efficient exsanguination process in DS group animals compared to PB group. However, it is important to note that various other factors might also influence heme iron content. Therefore, further research and analysis might be necessary to establish a definitive relationship between stunning methods and heme iron content.

Conclusion

The current study underscores the importance of considering stunning methods in the iron composition. While there was no significant difference in total iron content, the variance in heme iron content between stunning methods warrants further investigation. This research contributes to the broader understanding of factors influencing meat quality and might have implications for the meat processing industry and dietary considerations.

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