

INVESTIGATION OF PROTEOMIC CHANGES AND QUALITY OF SHEEP (OVIS ARIES) MEAT DUE TO ELECTRICAL STUNNING

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Abstract – The present study investigates changes in bleeding efficiency, physicochemical characteristics, and muscle proteome of sheep subjected to either electrical stunning and slaughtering or slaughter without any stunning (halal). Higher ($P < 0.05$) pH, water holding capacity, cooking loss and Warner-Bratzler shear force was observed in stunned sample, where as higher ($P < 0.05$) blood loss and Hunter lab a* values were observed in non-stunned meat. The 2DE gel analysis detected approximately 377 protein spots in which 243 (119 up regulated and 124 down regulated) protein spots were significantly ($P \leq 0.05$) altered with a fold change ratio $\geq 1.5/\leq 1.5$. The in-gel digestion and MALDI-TOF/TOF MS analysis of statistically significant protein spots revealed 35 non-redundant proteins out of which 26 were up-regulated and 9 were down-regulated. Biological information such as pathways, protein classes, cellular components, biological processes, and molecular functions was obtained by PANTHER analysis. Our findings demonstrated that slaughtering of sheep without stunning resulted in changes of the abundance of proteins involved in catalytic, structural, and stress related processes. This understanding of protein alterations in sheep slaughtered with and without stunning have the potential to act as possible signature for animal welfare index.

Key Words – electrical stunning, proteomics, halal

I. INTRODUCTION

In the meat production chain, slaughtering plays a crucial role in animal welfare, meat quality and safety (1). Meat consumers are increasingly demanding that animals be reared, handled, transported and slaughtered using humane practices (2). Animal welfare can be linked to carcass and meat quality. It is desirable to render an animal unconscious before it is slaughtered in order to eliminate pain, discomfort and stress from the procedure. Stunning is making an animal unconscious and insensible to pain before neck cut and also minimizes struggling through a more accurate and speedy slaughtering process (3).

Pre-slaughter stunning is compulsory according to EU Council Directive 93/119 (4). Due to widespread slaughter of animals using stunning, Muslim population with obligation to consume only halal (meaning permissible) food raised doubts over stunning methods (5) concerned about possible reduction in blood loss following the pre-slaughter stun.

The application of electrical stunning is a common practice in sheep industry. Bager *et al.* (6) and Cook *et al.* (7) have demonstrated that when done successfully, the additive effects of electrical stunning and exsanguinations minimize the time to permanent loss of brain function. Biochemical changes that occur upon slaughter have dramatic influence on meat quality and further meat processing steps. Stunning can have profound effect on meat quality. Although numerous studies have investigated the impact of stunning on welfare indicator and meat quality in sheep (8, 9, 10), the reports on relationship between stunning and muscle proteome in sheep are not available. Proteomics offers platform to understand the biochemical basis of meat quality in relation to post-mortem muscle metabolism and pre-slaughter handlings (11). Hence, this work was conceptualized to unravel the effects of slaughtering with stunning and slaughtering without stunning on

bleeding parameters, physicochemical qualities, color indices, and proteome profile of *Longissimus thoracis et lumborum* muscle in Nellore sheep.

II. MATERIALS AND METHODS

Eighteen months old Nellore sheep were separated into two group (n = 30 each) for pre-slaughter handling. In the first group, the sheep were slaughtered without previous stunning (NST). The second group was electrically stunned by the head only method at 1 A for 3 s (ST). Immediately after stunning, the sheep were slaughtered using conventional procedures. Post slaughter, blood volume was measured after 3-4 minutes bleeding. After dressing the carcasses were chilled for 24 hours to facilitate the completion of rigor-mortis. The *Longissimus thoracis et lumborum* muscle was separated from other muscles and used for current experiment. Raw meat was evaluated for pH, water holding capacity (12) and instrumental color. Cooked meat cubes were evaluated for Warner-Bratzler shear force and cooking losses. Total muscle proteins were extracted, purified and separated using 2-Dimensional gel electrophoresis (2-DE) from pooled muscle sample using previously standardized protocol (13). The statistically significant protein spots were excised from gels and subjected for trypsin digestion. The protein identification was established using MALDI-TOF/TOF MS (14). Biological information such as pathways, protein classes, cellular components, biological processes, and molecular functions was obtained by PANTHER analysis. The overall experiment was replicated on three separate occasions. Statistical analysis was performed with the paired t-test using SPSS (SPSS version 13.0 for windows; SPSS, Chicago, IL, USA).

III. RESULTS AND DISCUSSION

Results of bleeding efficiency, physicochemical characteristics and instrumental color are summarized in Table 1. The mean blood volume was higher ($P<0.05$) in non-stunned (NST, 1.16) compared with stunned (ST, 1.03) sheep, however, no ($P>0.05$) difference was found in bleeding efficiency and this agrees with findings of Khalid et al. (15). The mean pH was found to be higher ($P<0.01$) in ST (5.87) in contrast to NST (5.60). These pH values are within the range considered normal for sheep meat ranging 5.56 to 5.78 (16, 17). Significantly higher water holding capacity ($P<0.05$) and cooking loss ($P<0.01$) was found in ST compared to NST. Similarly, higher drip loss was observed in stunned compared to non-stunned sheep (10, 18). Warner Bratzler shear force was lower ($P<0.01$) in NST (16.99 N) relative to ST (16.15 N). Similar report with tendency for the meat from non-stunned lambs to be more tender than that from electrically stunned lambs has been found (19). The ST meat samples had Hue value of 0.71 ($P<0.01$) compared with 0.56 in NST. These results agree with previous findings by Linares et al. (10) who showed that meat from un-stunned lamb was darker (lower L^* value) than that of stunned. The Hunterlab color values obtained in the present study agrees with previous findings (20).

Table 1 Bleeding parameters, physicochemical qualities and color indices of Stunned (ST) and Non stunned (NST) muscle from sheep

Item	Stunned	Non-Stunned
Live weight	32.30±1.24	34.23±0.99
Blood Volume (l)*	1.03±0.01	1.16±0.04
Bleeding efficiency	3.20±0.12	3.39±0.05
pH**	5.87±0.02	5.60±0.03
Water Holding Capacity*	25.67±2.33	18.33±2.03
Cooking loss**	42.67±0.67	35.50±0.85
WBSF**	16.99±1.64	16.15±0.77
Hunterlab L*	21.72±1.43	19.53±0.59
Hunterlab a*	14.82±0.54	17.42±0.34
Hunterlab b*	12.94±0.88	11.25±0.76
Hue**	0.71±0.05	0.56±0.03
Chroma	20.01±0.53	20.86±0.63

* Significant at ($P < 0.05$)

** Significant at ($P < 0.01$)

The 2-DE analysis detected approximately 377 protein spots in which 243 (119 up-regulated and 124 down-regulated) protein spots were significantly ($p < 0.05$) altered with fold change ratio $\geq 1.5/\leq 1.5$. The protein identification established using MALDI-TOF/TOF analysis revealed 35 non-redundant proteins out of which 26 were up-regulated and 9 were down-regulated. Differential expression profile of protein location in representative 2DE gel is shown in Fig. 1. Variation in expression of myosin, actin and troponin was seen between ST and NST samples. Myosin is the most abundant protein and contributes to the structure and tensile strength of meat (21). The actin and actin-bundling proteins have been reported to play a significant role in muscle contraction (22). Expression of Heat shock protein has been linked to stress by various researchers (23). Biological information such as pathways, protein classes, cellular components, biological processes, and molecular functions was obtained by PANTHER analysis. In biological function analysis, the set of 35 proteins identified were found to be involved in various functions such as transportation, complement cascade, inflammatory response, cell proliferation, etc. These protein sets were part of cell part, organelle, and macromolecular complex.

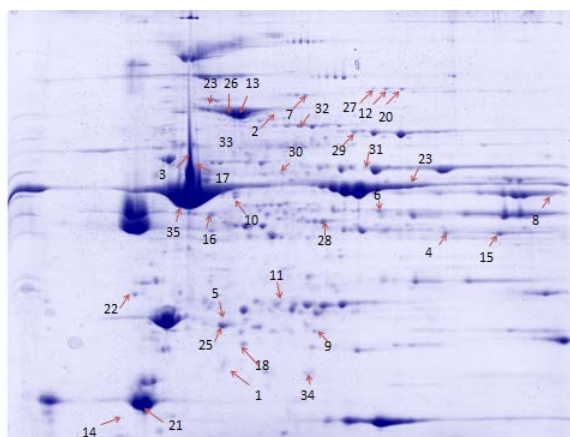


Figure 1. Representative 2-DE image indicating differentially expressed proteins

IV. CONCLUSION

The study demonstrated significant variation in bleeding efficiency, textural and color parameters between sheep meat produced by slaughtering sheep with or without stunning. Proteome characterization using 2DE and MALDI-TOF/TOF MS identified important proteins/ peptides that correlates with meat texture and pre-slaughter stress. This study provides new insight into the possible variation in proteome expression which explains differences in degree of protein denaturation, ultimate meat quality, and animal welfare parameters between animals slaughtered using traditional halal method and humane slaughter using electrical stunning. Further research is needed to validate the relationship that exists between proteome profile observed with other meat quality traits.

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REFERENCES

1. Sabow A. B., Zulkifli, I., Goh, Y.M., Ab Kadir, M.Z.A., Kaka, U., Imlan, JC, et al. (2016) Bleeding Efficiency, Microbiological Quality and Oxidative Stability of Meat from Goats Subjected to Slaughter without Stunning in Comparison with Different Methods of Pre-Slaughter Electrical Stunning. *PloS ONE*, 11(4): e015.
2. Appleby, M.C., & Hughes. B.O. (1997). *Animal Welfare*. Wallingford: CAB International, U.K.
3. Aberle, E. D., Forrest, J. C., Gerrard, D. E., Mills, E. W., Hedrick, H. B., Judge, M. D., & Merkel, R. A. (2010). *Principles of meat science*. Dubuque, IA: Kendall/Hunt Publishing Company.
4. EU council Directive 93/119. (1993). On the protection of animals at the time of slaughter or killing. *Official Journal of European Community*, 1340/21, 1993.
5. Nakyinsige, K., Fatimah, A., Aghwan, Z.A., Zulkifli, I., Goh, Y.M., & Sazili, A.Q. (2014). Bleeding efficiency and meat oxidative stability and microbiological quality of New Zealand White rabbits subjected to halal slaughter without stunning and gas stun-killing. *Asian-Australia Journal of Animal Sciences*, 27: 406–413.
6. Bager, F., Braggins, T.J., Devine, C.E. & Graafhuis, A. E. (1992). Onset of insensibility at slaughter in calves: effects of electroplectic seizure and exsanguination on spontaneous electrocortical activity and indices of cerebral metabolism. *Research in Veterinary Science* 52, 162-173.
7. Cook, C. J., Maasland, S. A., Devine, C. E., Gilbert, K. V., & Blackmore, D. K. (1996). Changes in the release of amino acid neurotransmitters in the brains of calves and sheep after head-only electrical stunning and throat cutting. *Research in Veterinary Science*, 60(3), 255–261.
8. Vergara, H., Linares, M. B., Berruga, M. I. & Gallego, L. (2005). Meat quality in suckling lambs: effects of pré-slaughter handling. *Meat Science*, 69(3); 473-478.
9. Velarde, A., Gispert, M., Diestre, A. & Manteca X. (2003). Effect of Electrical Stunning on Meat and Carcass Quality in Lambs. *Meat Science*, 63, 35-38.
10. Linares, M. B., Bómez, R., & Vergara, H. (2008). Cortisol and catecholamine levels in lambs: effects of slaughter weight and type of stunning. *Livestock Science*, 115(1), 53-61.
11. Amid, A., Abu Samah, N., & Yusof, F. (2012). Identification of troponin I and actin, alpha cardiac muscle 1 as potential biomarkers for hearts of electrically stimulated chickens, *Proteome Science*, 10/1.
12. Wardlaw, F. B., Maccaskill, L. H. & Acton, J C. (1973). Effect of postmortem muscle changes in poultry meat loaf properties. *Journal of Food science* 38; 421-424.
13. Kiran et al., Kiran, M., Naveena, B.M., Reddy, K.S., Shahikumar, M., Reddy, V.R., Kulkarni, V.V., Rapole, S. & More, T.H. (2016). Understanding tenderness variability and ageing changes in buffalo meat: biochemical, ultrastructural and proteome characterization. *Animal*. 10(6):1007-15.
14. Shevchenko, A., Tomas, H., Havlis, J., Olsen, J.V. & Mann, M. (2006). In-gel digestion for mass spectrometric characterization of proteins and proteomes. *Nature Protocols*, 1(6):2856-60.
15. Khalid, R., Knowles, T.G. & Wotton, S.B. (2015). 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. *Meat Science*, 110: 15–23.
16. Hopkins, D.L. & Mortimer, S.I. (2014). Effect of genotype, gender and age on sheep meat quality and a case study illustrating integration of knowledge. *Meat Science*, 98(3):544-55.
17. Sen, A.R., Naveena, B.M., Muthukumar, M. & Vaithiyanathan, S. Colour, myoglobin denaturation and storage stability of raw and cooked mutton chops at different end point cooking temperature. (2014). *Journal of Food Science Technology*, 51(5), 970–975.
18. Önenç, A. & Kaya, A. (2004). The effects of electrical stunning and percussive captive bolt stunning on meat quality of cattle processed by Turkish slaughter procedures. *Meat Science*, 66(4): 809-815.
19. Vergara, H. & Gallego, L. (2000). Effect of electrical stunning on meat quality of lamb. *Meat Science*, 56, 345-349.
20. Sañudo, C., Enser, M.E., Campo, M.M., Nute, G.R., María, G., Sierra, I.E. & Wood, J. D. (2000). Fatty acid composition and sensory characteristics of lamb carcasses from Britain and Spain. *Meat Science*, 54(4): 339-346.
21. Marino, R., Albenzio, M., Malva, A.D., Santillo, A., Loizzo, P. & Sevi, A. (2013) Proteolytic pattern of myofibrillar protein and meat tenderness as affected by breed and aging time. *Meat Science*, 95: 281–287.
22. Xue, M., Huang, F., Huang, M. & Zhou, G. (2012). Influence of oxidation on myofibrillar proteins degradation from bovine via μ -calpain. *Food Chemistry*, 134: 106–112.
23. Chulayo, A. Y. & Muchenje, V. (2015). A balanced perspective on animal welfare for improved meat and meat products: A review. *South African Journal of Animal Science*, 45(5), 452–469.