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Effect of Electrical Stimulation on Structural Characteristics of Spent Rabbit Carcass

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Abstract: A study was carried out to evaluate the effect of low voltage electrical stimulation (110 and 200 V) on the structural characteristics viz, fFiber diameter, sarcomere length, myofibrillar fragmentation index (MFI), shear force values of spent rabbit carcasses. A significant decrease ($P<0.01$) in fiber diameter and increase in sarcomere length was observed on ES at 1 hr, whereas no significant ($P> 0.05$) difference at 24 hrs. MFI decreased significantly ($P<0.01$) with increase in voltages of ES. A significant ($P < 0.01$) decrease in shear force value was recorded between control and two treatment groups. However, there was no significant difference between the two treatment groups. Histological studies revealed physical disruption of myofibrils and contracture bands in ES groups. No significant difference was observed between the two voltages, but ES was effective in improving the tenderness and other quality parameters of spent rabbit carcasses compared to control.

Key words: Electrical stimulation, Sarcomere length, Fiber diameter, Myofibrilar fragmentation index.

INTRODUCTION

The meat supply situation in India remains critical in spite of the relatively large animal population, mainly because of the existing religious customs. Chicken, mutton/ Chevon have found a major part in Indian diets since a prolonged period of time because of its acceptability by people from all communities. But the false propaganda on the ill effects of consumption of these meats has made the consumers more selective and to look for alternative source of animal protein. Short cycle production animals like rabbit can be a better alternative to conventional food animals and has the advantage of its family sized carcass and the absence of religious obstacles for its consumption [1].

Intensive commercial rabbit rearing has acquired greater interest in developing countries like India, where consumers prefer meat with high palatability, desirable meat: bone ratio, high biological value, low cholesterol, calories and sodium, which are inherent property of rabbit meat. Due to rapid growth of rabbit industry in India for meat and fur, marketing of spent rabbits after their economic breeding age poses serious concerns to the producers because of its toughness and lower eating quality. Tenderness is one of the main attributes that consumers and retailers consider a primary component of palatability and thus a measure of the quality of meat [2]. Advanced age and or increased physiological maturity are associated with toughness thereby decreasing the value of meat from such animals. A consistent method for improving tenderness of such spent carcasses to a more acceptable level would increase their retail value and thereby increasing the marketing opportunities [3].

Since Stone Age man has been endeavoring to tenderize meat artificially by different methods like beating, cutting, wrapping in papaya leaves, use of proteolytic enzymes and aging. In the last three decades electrical stimulation (ES) has received considerable attention and has been applied in the red meat industry for improved meat quality characteristics, decreased processing time and a possible decrease in microbial cross contamination [4]. ES reportedly speeds up the onset of rigor, lessens aging and produces more tender meat in beef and lamb [5].

The structural changes are assessed by measuring the fiber diameter since the average fiber size was correlated to sensory panel tenderness and shear force measurements [6]; sarcomere length is related to the ageing response in that, muscles with shorter sarcomere lengths had a greater ageing response [7] and myofibrillar fragmentation index (MFI) is important because, it is a measure of structural change in native myofibrils [8,9]. Although a great deal of research has been carried out into the effect of ES in lamb and beef carcasses [10 - 12],

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very little has been conducted to study the effect of low voltage ES in improving the quality of spent rabbit carcass. In view of the above, the present study was undertaken to study the effect of ES (110 and 220 V) on the structural characteristics of spent rabbit carcass for improvement of its acceptability.

MATERIALS AND METHODS

The tenderizing effect of electrical stimulation on spent rabbit carcasses using two different voltages (110 and 220 volts) was assessed.

Experimental Design: Twenty spent rabbits over 2½ years with live weight range of 1.5 - 2.5 kg were used for this study. The rabbits were divided into two equal groups (Groups I and II), each consisting of 10 rabbits. Group I and Group II animals were subjected to two different treatments viz. electrical stimulation with 110 V and 220 V, respectively. The rabbits were received one day prior to slaughter, fed with pellet feed, *ad libitum,* hay and water in the lairage. Feed was withdrawn 12 hours prior to slaughter and ante mortem inspection was conducted before slaughtering. The animals were slaughtered and dressed as per the standard procedure with official permission from *Institutional Animal Ethics Committee* and a detailed postmortem examination of the carcasses were conducted and then the carcasses were split along the spinal column into two identical halves leaving the neck with the left half. The left half was used for electrical stimulation and the right half was kept as a control.

Electrical Stimulation: Both the halves were suspended from the Achilles tendon with the help of a stainless steel hook and the left half was subjected to electrical stimulation within 20 minutes after exsanguinations with the help of an "Electrostim" (H.S. Trading Company, Madras), which can deliver a current of four different voltages (110, 220, 330 and 440 V) and produce 0 to 99 (count) DC square wave pulses of 0 to 99 x 0.1 second pulse width. The positive (anode) electrode was inserted at the distal end of the *m.biceps femoris* and *m. semitendinosus*, the negative (cathode) electrode was inserted into *m.brachiocephalicus*. Group I carcasses were stimulated with 110 V, 10 pulses of 6 second pulse width for a total period of 90 seconds and Group II carcasses were stimulated with 220 V, 10 pulses of 6 second pulse width for a total period of 90 seconds.

Structural Parameters

Fibre Diameter: Fibre diameter of both stimulated and control samples were assessed according to the method outlined by Jeremiah and Martin [13] at 1 hr and 24 hrs postmortem. A five gram sample of *longissimus dorsi* muscle was cut into small pieces and homogenised in a blender at a low speed for two 15 second period interspaced with a 5 second resting interval, in a 30 ml solution containing 0.25 M sucrose and 1 M ethylene diamine tetra acetic acid (EDTA) to form slurry. One or two drops of the slurry was then transferred on to a microscopic slide and covered with a cover slip. The suspension was examined under low power in a light microscope fitted with 10x objective and 8x eye piece equipped with calibrated micrometer. Muscle fiber diameter was measured as the mean cross-sectional distance in micrometers between the exterior surfaces of the sarcolemma of 20 randomly selected muscle fibers.

Sarcomere Length: Sarcomere length was determined as per the method described by Cross *et al.* [14]. Five grams of meat sample was blended with 35 ml of 0.25 M sucrose solution for one minute in a blender at low speed. Immediately after blending, a drop of the slurry containing the fiber fragments were transferred on to a microscopic slide and covered with a cover slip. The suspension was examined under a microscope using an 8x eye piece with a calibrated micrometer under oil immersion objective. Sarcomere length was measured as the mean length of 10 Sarcomere on 25 randomly selected myofibrils.

Myofibrillar Fragmentation Index (MFI): The myofibrillar fragmentation index was determined in stimulated and control specimens, frozen *longissimus dorsi* samples at 24 hrs as per the method followed by Davis *et al.[15]* with slight modifications. After removal of epimyseal tissue and subcutaneous fat 7 mm cubes were made from the frozen meat sample. Ten grams of such cubes were transferred to a 250 ml virtis flouted glass homogenization cup containing 50 ml of cold 0.25 M sucrose and 0.02 M potassium chloride solution. The muscle cubes were allowed to thaw for 5 minutes, with two virtis macro stainless steel blades aligned and positioned 1 mm below the surface of the solution and set in a reverse position, the sample was homogenized for 40 sec. at full speed. The homogenate was filtered through a pre weighed 250 μm pore stainless steel wire cloth screen in the filtration unit. The homogenate was stirred with a glass rod to hasten the filtration process. The resulting fraction of muscle fragments greater than 250 μm in size collected on the

screen was blotted with Whatman No.3 filter paper. The weight of the sample with the screen was taken after 40 minutes of drying at 40° C. The fragmentation index was obtained by multiplying the weight of the fraction remaining on the screen (in grams) by 100.

hear Force Value (SFV): The thigh muscles of both control and stimulated halves were used for assessment of cooked shear force value using Warner-Bratzler meat shear (G.R. Electric Manufacturing Company, Manhattan, U.S.A.) at 24 hrs postmortem. The legs were cooked at 15 1bs pressure for 15 minutes and the cooked samples were cooled in a refrigerator and 1 inch core of 1.25 cm diameter was collected from the cooked and cooled samples. Three readings were taken from each core and the average of the three readings were recorded as the mean shear force value, expressed in kg/cm^2.

Taste Panel Studies: Organoleptic assessment of 24 hrs stored samples of both control and stimulated muscles were conducted. Cubes of 1.5 cm were cut from leg muscles in sufficient numbers and were cooked at 15 1b pressure for 15 minutes. Coded samples were served to the trained panelists drawn from the staff of the Department of Meat Science and Technology, Madras Veterinary College. The members were provided with a score card having a nine point hedonic scale to assess the appearance, flavour, juiciness, tenderness and overall palatability. Data generated from the study were analyzed for statistical significance as per the methods outlined by Snedecor and Cochran [16].

RESULTS

Fiber Diameter: Fiber diameter decreased ($P < 0.01$) at 1hr in the stimulated group than the control group, with no significant difference between both groups. Also, fiber diameter at 24 hrs did not reveal any significant difference between both groups (Table 1).

Sarcomere Length: An increased ($P < 0.01$) sarcomere length was observed in 220 V treatment group only at 1 hr postmortem. However, there was no significant difference in the sarcomere length between control and treatment groups at 24 hr (Table 1).

Myofibrillar Fragmentation Index: A decreased ($P < 0.01$) myofibrillar fragmentation index was observed in the in 220 V groupas compared with the control group, but no significant decrease was observed between control and 110 V treatment groups (Table 1).

Table 1: Mean±S.E. Values of Structural Characteristics of Control and Stimulated Rabbit Carcasses (110 AND 220 V)

			Stimulated	
Parameter	Time	Control	110 V	220 V
MFI	0 h	879^a±22.3	861^a±36.3	725^b±17.3
SFV	0 h	4.17^a±0.080	2.67^b±0.279	2.58^b±0.195
Fibre diameter (µm)	1 h	75.49^a±2.721	62.94^b±3.451	61.99^b±2.662
	4 h	64.69±2.553	53.47±3.579	55.83±3.578
Sarcomere Length(µm)	1 h	1.52^a±0.037	1.71^{ab}±0.103	1.87^b±0.045
	24 h	1.84±0.032	1.94±0.096	1.98±0.033

Means bearing different superscript between rows differ significantly ($P<0.01$)

Table 2: Mean±S.E. Values of Taste Panel Scores of Control and Stimulated Rabbit Carcasses (110 AND 220 V)

		Stimulated	
	Control	110 V	220 V
Flavour	6.14^a±0.174	6.70^{ab}±0.233	7.44^b±0.261
Juiciness	6.85^a±0.105	6.08^b±0.207	5.90^b±0.215
Tenderness	6.08^a±0.109	7.02^b±0.203	7.57^b±0.183
Overall Acceptability	6.35^a±0.064	6.60^{ab}±0.125	6.97^b±0.102

Means bearing atleast one common superscript in each row do not differ Significantly

Shear Force Value: There was a decrease t ($P < 0.01$) in shear force value between control and two treatment groups. However, there was no significant difference between the two treatment groups (Table 1).

Histological Studies: Stimulated muscle samples showed contracture bands throughout the myofibre with stretched sides on either side of the contracture bands.

Correlation Studies: Results of correlation study indicated that fiber diameter was negatively and insignificantly correlated with sarcomere length. Shear force value was positively correlated with fibre diameter and myofibrillar fragmentation index, whereas sarcomere length is negatively correlated with shear force value (Table 1).

Taste Panel Studies: There was an observed increased ($P < 0.01$) in flavour, tenderness and overall palatability scores and a decrease in juiciness scores between control and stimulated (220 V) group. However, there was no significant difference observed between the two treatments (Table 2).

DISCUSSION

In the present study fiber diameter markedly decreased 1hr post Electric stimulation with nosignificant difference between 110 and 220 Vtreatment groups. Moreover, the diameter 24 h did not reveal any significant difference between treatment groups and control. Thee results are in congruent with the findings of Borpuzari [17] and Kuttinarayanan [18] who observed significant ($P < 0.01$) decrease in fibers diameter in samples of electrically stimulated sheep carcasses. The absence of significant difference in the values obtained for fiber diameter in two treatment groups showed that stimulation with 110 V did not commensurate with risks involved in higher voltage.

In this study, obvious increase was recorded in sarcomere length of 220 V treatment group only at 1 hr postmortem with no significant difference between control and treatment groups at 24 hrs. However, increased sarcomere lengths in stimulated samples were observed in beef [19] pork [20] and chicken [21] carcasses.

Marked decrease was observed in the myofibrillar fragmentation index between control and 220 V group herein, but no significant decrease was observed between control and 110 V treatment groups. The results clearly indicted the efficacy of electrical stimulation in bringing about appreciable amount of tenderness. Salm *et al.* [22] and Borpuzari [17] observed a decrease in myofibrillar fragmentation index due to electrical stimulation in beef and sheep carcasses, respectively. The decrease in myofibrillar fragmentation index due to electrical stimulation may be due to enhanced degradation of myofibrillar proteins, alpha actinin and troponin – T.

Significant decrease in shear force value was recorded in this study between control and two treatment groups. However, there was no significant difference between the two treatment groups. The degradation of connective tissue and denaturation of myofibrillar proteins could be due to enhanced postmortem glycolysis caused by electrical stimulation leading to significant reduction in shear force value [10, 18, 21 and 24]. The absence of significant difference between two treatment groups on the shear force value clearly indicated that there was no enhancement in tenderness with increase in voltage.

Stimulated muscle samples herein showed contracture bands throughout the myofibre with stretched sides on either side of the contracture bands. Also, Savell *et al.* [23] and McKeith *et al.* [25] observed similar contracture bands and physical disruption of myofibrils. Sorinmade *et al.* [26] surmised that the contracture bands caused super stretching of the myofibrils leading to tearing and physical disruption on either side of the contracture bands. No such changes have been observed in control muscle samples. Histological sections with haemotoxylin and eosin stain revealed physical disruption, absence of I-band or A-bands, fragmentation of myofibrils and complete disorganization of the electrically stimulated muscle tissue. Similar observations were also made by Sorinmade *et al.* [26] and Fabiansson and Laser Reutersward [27].

The significant increase in flavour, tenderness and overall palatability scores and a decrease in juiciness scores in this study were in line with the finding of Calkins *et al.*[28] between control and stimulated (220 V) group. However, there was no significant difference observed between the two treatments. Smith *et al.* [5] observed high flavour ratings for electrically stimulated beef samples and attributed it to differences in the concentration of creatine phosphate, adenine nucleotides and their derivatives caused by electrical stimulation.

It can be concluded that spent rabbit carcasses could be electrically stimulated with 110 V to improve the tenderness and other quality parameters, without much risk involved in the operation.

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