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THE INFLUENCE OF SLAUGHTER TECHNIQUE AND HISTOLOGICAL TREATMENT ON MUSCLE FIBRE DIAMETER OF LOW AND HIGH pH_1 PORK MUSCLE

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OPSOMMING: DIE INVLOED VAN SLAGMETODE EN HISTOLOGIESE BEHANDELING OP SPIERVESELDEURSNEE VAN LAE EN HOË pH_1 VARKSPIER

'n Proef was uitgevoer om die invloed van twee slagmetodes en drie metodes van spierfiksering op die mikrostruktuur van die varkspier te bepaal. Die spier van varke wat met die penpistool bedwelm is het 'n baie laer gemiddelde pH_1 (5,50) gehad as die varke wat nie bedwelm was nie (6,70). Statisties betekenisvolle verskille in spierveseldeursnee is gevind, wat aan die slagmetode toegeskryf kon word. "Reuse vesels" soos deur Cassens, Cooper & Briskey (1969) beskryf is, is gevind in spiermonsters van varke wat volgens albei metodes geslag is.

SUMMARY

An experiment was conducted to assess the effect of two slaughter methods and three methods of muscle fixation on the microstructure of pig muscle. The muscle from the pigs stunned with a captive bolt had a much lower average pH_1 (5,50) than the unstunned pigs (6,70). Statistically significant differences in fibre diameter due to method of slaughter and method of fixation were recorded. "Giant fibres" as described by Cassens, Cooper & Briskey (1969) were found in muscle samples taken from pigs slaughtered by both methods.

The pale, soft and exudative (P.S.E.) phenomenon in pigs is caused by a rapid fall in muscle pH ($pH_1 < 6,0$ at 45 min.) *post mortem* while muscle temperature is still at a reasonably high level ($> 35^{o}C$) (Briskey, 1964). In South Africa it has been established that stunning pigs by captive bolt gives rise to low muscle pH_1 values *post mortem*. A recent country wide survey of methods of slaughter indicate that percentages of pH_1 values lower than 6,0 as high as 72% can be expected when the captive bolt method is used (Klingbiel & Naudé, 1971).

To investigate certain traits of low pH_1 and normal musculature a low pH_1 was induced in the *M. longissimus dorsi* of a group of pigs stunned by captive bolt pistol and compared with normal muscle obtained from pigs exsanguinated without stunning. Muscle fibre size of these two groups was studied following different processing procedures. "Giant fibres" were observed in both low pH_1 and normal musculature. According to the literature (Cassens *et al.* 1969) "Giant fibres" are only associated with P.S.E. or low pH_1 muscles.

Procedure

Eight Landrace x Large White crossbred pigs fed a high level of protein ration (16% protein) were divided into two groups with a mean carcass weight of 65 kg for each group. One group was stunned by captive bolt pistol to induce low pH_1 musculature while the other group was exsanguinated without stunning. The pH and temperature of the *M. longissimus dorsi* at the level of the 11th, 12th and 13th thoracic vertebrae were determined 45 min. *post mortem*. Twenty-four hours *post mortem* a section of the same muscle was taken at the same position. All specimens for processing were taken in the same area of the muscle and were cut transversely to the longitudinal axis of the muscle fibres and fixed in 10% neutral Formalin, Carnoy's and Zenker's according to the prescribed formulae (Luna, 1968). All fixed specimens were processed and embedded in paraffin wax with 5% ceresine (M.P. 57^{o}–$60^{o}C$). Sections were cut at 7 µm and stained with Delafield's haematoxylin and counterstained in eosin.

Specimens used for cryostat sectioning were immersed in liquid nitrogen prior to deep freeze storaging at $-20^{o}C$. These were sectioned at 10 µm and stained with Mallory's Triple Stain.

Homogenized specimens were prepared according to the method of Hegarty & Naudé (1970) for immediate measurement of the diameter of 100 muscle fibres.

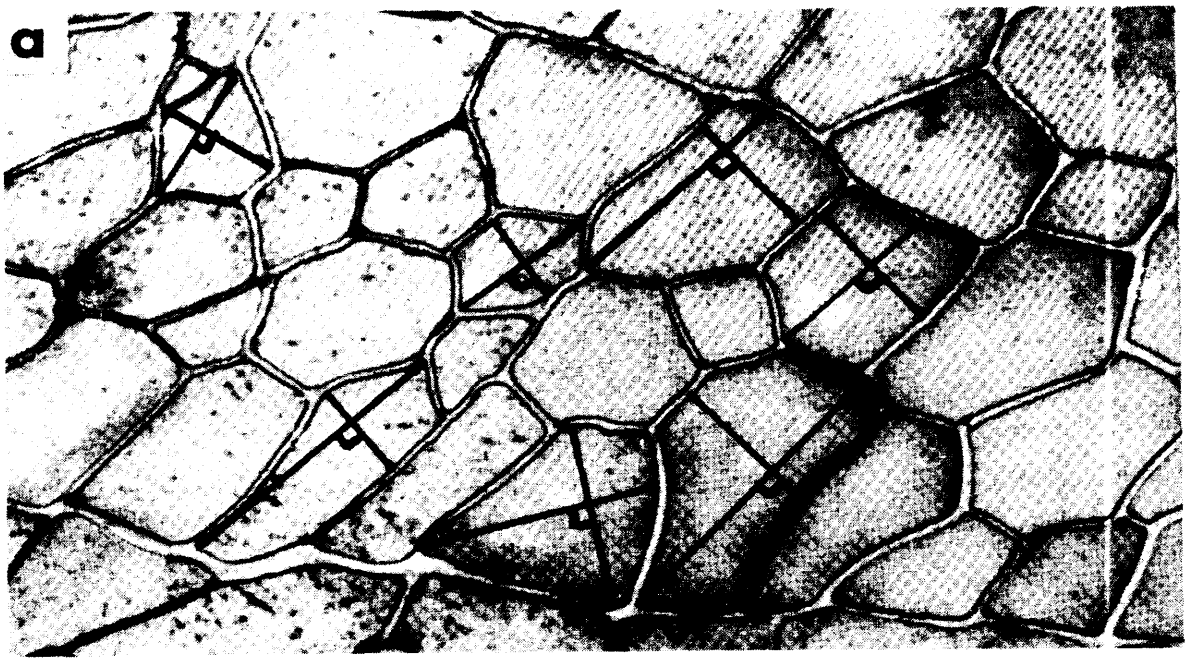
One hundred muscle fibres from samples prepared by the former four methods were measured per slide on a Reichert Visopan at a magnification of 500x. Transverse sections were measured across their widest long axis and the widest short axis perpendicular to the widest long axis (Plate 1a).

An analysis of variance on the results was carried out according to the method of Guenther (1964).

Results and Discussion

Effects of slaughtering method

In Table 1 the differences between low and high pH_1 musculature is clearly illustrated showing that the pH_1 of the exsanguinated pigs is 6,7 while that of the pigs



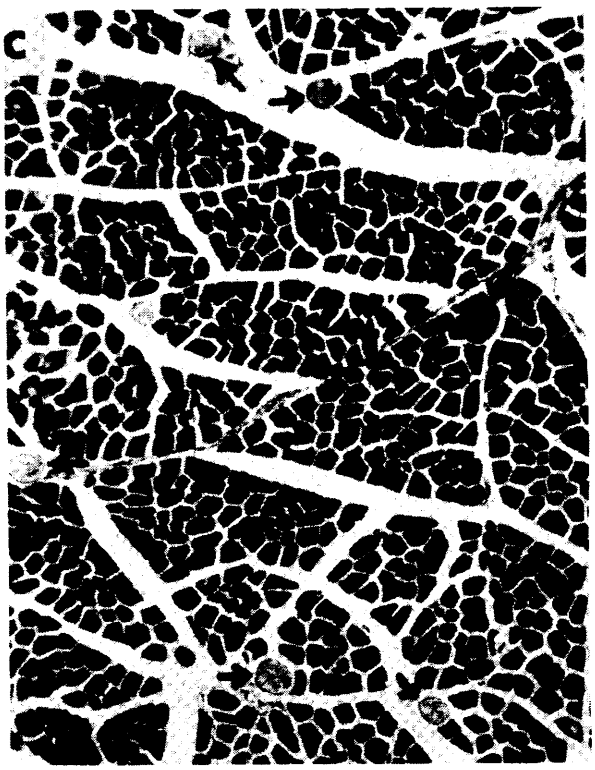


Plate I. —*a. The axes along which cross-sectional measurements were taken (X 840).*
b. Transudation of the cell content into extracellular spaces as indicated by arrows (X 260).
c. A number of "Giant fibres" as indicated by arrows (X 12).

Table 1

Muscle fibre diameter of low pH_1 and normal pig M. longissimus dorsi (µm)

	pH_1	Carcass Mass kg	No.	Carnoy's	10% Formalin	Zenker's	Cryostat	Homogenizer	Mean	"Giant Fibre" Mean
				1	2	3	4	5	6	
Exsanguinated	6,70	64,9	4	44,27	39,01	41,56	47,23	64,39	47,29	65,87 (n = 89)
				7	8	9	10	11	12	
Captive Bolt	5,50	64,4	4	38,08	35,05	39,95	40,08	54,93	41,62	71,93 (n = 63)
				13	14	15	16	17		
Mean				41,17	37,03	40,76	43,66	59,66		

$P < 0,01$ 6 : 12; 17: 13, 14, 15, 16; 5 : 1, 2, 3, 4; 11 : 7, 8, 9, 10; 5 : 11; 4 : 10

$P < 0,05$ 1 : 7; 14 : 15

stunned by captive bolt is 5,5. The analysis of variance emphasized that the method of slaughtering had a statistically significant ($P < 0,01$) effect on fibre size. This condition resulting from a low pH_1 in a muscle is also reflected in the appearance of the meat when cut and exposed (Naudé, 1972). Measurements on muscle fibre diameter (Table 1) showed a statistical significant difference for the means (6:12) at the $P < 0,01$ level for the two slaughter groups indicating a shrinkage in fibre size of the low pH_1 muscles.

The decrease in fibre diameter can be attributed to a poorer waterholding capacity of the myofibrillar proteins of the muscle fibres exposed to a rapid *post mortem* glycolysis (Carroll, 1971) resulting in the transudation of intracellular moisture into the intracellular spaces (Plate 1 b). Evidently this is the reason for the moist appearance of low pH_1 or P.S.E. pork on the exposed surface of muscles.

Effects of Laboratory processing

It is apparent that both the homogenizer (5:11) (Table 1) and the cryostat (4 : 10) treatments are sensitive enough to show differences between the two slaughter methods at the $P < 0,01$ level. Measurements on fibre diameter of specimens from both slaughter methods fixed in Carnoy's (1:7) show this sensitivity only at the 5% level of significance while the measurements on specimens fixed in 10% neutral Formalin (2:8) and Zenker's (3 : 9) show no statistical difference at all between the two slaughter methods. Shrinkage as such could also be due to the effects of laboratory processing. However, since all specimens were treated in the same manner the statistical differences in fibre diameter between the two groups can be attributed to the slaughter method and not to shrinkage during processing. No interaction could be shown between slaughter method and laboratory processing.

This decrease in muscle fibre diameter is all the more noticeable when comparing diameters of fibres fixed in Carnoy's, 10% Formalin and Zenker's on the one hand, with the unfixed fibres of cryostat sections and those of the homogenizer on the other hand (Table 1). Formalin material showed the greatest amount of shrinkage of the three fixatives used during laboratory processing which coincides with findings of other workers (Goldspink, 1961).

"Giant fibres"

During these investigations the presence of "giant fibres" as described by Cassens *et al*, (1969) were noted (Plate 1c). These fibres were identified morphologically but contrary to the findings of the above workers they were also observed in sections of muscles from animals not conforming to the characteristics of low pH_1 pork muscle. These morphologically different fibres occurred in both groups under study in comparable numbers (Table 1). Since they can only be identified in transverse section at this stage, measurements could only be made in that plane.

In the exsanguinated group the mean diameter of these fibres was 65,87 µm (n = 89) and in the captive bolt group, 71,93 µm (n = 63) for the three processing proced-

ures as well as cryostat sections. Owing to low numbers and because they do not appear in every section a statistical analysis was not attempted. The reason for the larger fibre diameter of these fibres in the low pH_1 group could not be explained but the resistance of these fibres to the influence of rapid *post mortem* glycolysis and also to the influence of laboratory processing can be inferred from these measurements and the fact that they retain their circular or slightly oval shape.

Conclusions

A low pH_1 in muscle can be induced by the slaughtering method. This low pH_1 is usually associated with P.S.E. musculature in pigs. Histologically this condition is exhibited by the statistically highly significant decrease in fibre size of the low pH_1 muscles compared to the other group. This decrease in fibre size can be ascribed to the greater degree of transudation of cellular fluid to the extracellular spaces of the muscle with a low pH_1 as compared to the muscle with a high pH_1. Of the fixatives used, 10% Formalin showed the greatest shrinking influence on the muscle fibres of the muscles from both groups, followed by Zenker's and Carnoy's respectively. The homogenizer and cryostat method are sensitive to differences in muscle fibre diameter induced by the slaughtering method in a highly significant way ($P < 0,01$).

"Giant fibres" are found to occur in both low pH_1 and normal musculature. It can be inferred from this study that "giant fibres" are not visibly affected by the slaughtering method or laboratory processing. Further investigation into the biochemical characteristics of these fibres is needed to clarify their specific resistance to changes as manifested by their relatively large size and round or oval shapes. In addition, they have a characteristic staining reaction in contrast to the irregular polygonal shapes and different staining reaction of the bulk of the muscle fibres.

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