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THE AGEING OF BRUISES
IN LAMBS

A Thesis presented in partial fulfilment
of the requirements for the
Degree of Doctor of Philosophy
at Massey University

Ronald Norman Thornton
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ABSTRACT

Bruising in lambs processed for human consumption is a significant economic problem. A reduction in the prevalence of bruises could be achieved relatively efficiently if their important places of occurrence could be identified by ageing these lesions with respect to the known times of occurrence of events of possible aetiological significance. To this end efforts were made to age experimental bruises in lambs by objectively assessing semi-quantitative histopathological data using a mathematical model based on Bayes' theorem of inverse probabilities, by enzyme histochemical and isoelectric focussing studies and by measurement of muscle pH.

The Bayesian method for objective histopathological ageing was developed and tested on data representing 178 bruises. It was successful in identifying bruises as either 1-20 hours or more than 24 hours old. The 'accuracy' with which a bruise of known age could be identified as such depended on the nature and number of tissue samples studied. The degree of 'confidence' with which an individual bruise of unknown age could be aged, however, depended both on the 'accuracy' of the method and on the relative number of bruises estimated to belong to each of the two age categories considered. In general a degree of 'confidence' of 80-90% can be expected in practice, and in this respect the performance of the Bayesian method is superior to that achieved by purely subjective means. A pilot survey involving 107 bruises collected from an export meat works established both the practical value of the objective ageing method and its superiority over alternative epidemiological approaches to the problem of utilising data pertaining to trucking times and holding times in meat works yards. Of the bruises studied, 60% were estimated to have been inflicted within the works, and 40% prior to arrival.

Enzyme studies on bruises aged 4-144 hours old revealed no detectable relationships between observed changes in either histochemical or isoenzyme activities and bruise ages. In light of contradictory published results pertaining to other types of wounds, this lack of success was thought to reflect the relatively mild nature of the tissue reaction in bruises.
Statistically significant relationships could not be demonstrated between absolute or relative muscle pH and the ages of bruises from 4-48 hours old.

A newly recognised condition of 'subcutaneous haemorrhagic speckling' in the carcase adipose tissue of young lambs processed for human consumption was investigated. From histopathological and epidemiological evidence, the primary cause of the lesions was shown to be electrical stunning. However, secondary aetiological factors were proposed as having influenced the prevalence and severity of lesions. Attempts to elucidate the pathogenesis of 'speckling' with the intention of formulating a rational approach to its prevention were unsuccessful.
ACKNOWLEDGEMENTS

This research was conducted using the facilities of the Department of Veterinary Pathology and Public Health at Massey University. I am grateful to Professor B.W. Manktelow for the opportunity of undertaking the study and to others who have offered assistance over the past years. In particular I would like to thank Dr R.D. Jolly and Professor D.K. Blackmore for their guidance and unfailing encouragement throughout.

Invaluable advice on mathematical aspects of the thesis was provided by Professor R.E. Munford of the Department of Physiology and Anatomy. The late Dr R.E. Harris of the Department of Veterinary Clinical Sciences helped plan and execute the epidemiological section of the study on 'subcutaneous haemorrhagic speckling'.

Paraffin sections for light microscopy were prepared by Mrs P.M. Slack and Miss S.L. Malloch, both of the Department of Veterinary Pathology and Public Health. Photographs were processed with the help of Mr T.G. Law. Willing assistance in all practical aspects of animal handling was given by Mr A.T. De Cleene and Mr C.K. Barnett. The thesis was typed by Miss S.J. Shirriffs.

The investigation into 'subcutaneous haemorrhagic speckling' and the pilot bruise survey were conducted at the Hawke's Bay Farmers Meat Co. Ltd and Borthwicks-CWS, Feilding respectively. I am grateful to the employees of both of these companies for their helpfulness in these aspects of the study. Of particular note was the cooperation and enthusiasm of Messrs N. Marsden and P. Dingeman of the former company.

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Bruise 24 hours old. Strongly positive alkaline phosphatase staining of capillary endothelium. Neutrophils are also strongly positive. x 125

Bruise 24 hours old. Variable alkaline phosphatase staining of infiltrating macrophages. x 500

Alkaline phosphatase zymogram of bruised muscle, small intestine and liver after isoelectric focussing at 25 watt constant power for 1.5 hours in PAG with ampholine buffer range pH 3.5-9.5. The gel was incubated in aqueous substrate medium for 30 minutes at 37°C.

Bruise 144 hours old. Apparently positive leucine aminopeptidase staining of a necrotic muscle fibre, actually due to infiltrating macrophages. x 320

Bruise 4 hours old. Positive leucine aminopeptidase staining in the absence of infiltrating macrophages. x 320

Bruise 144 hours old. Moderate leucine aminopeptidase staining of fibroblasts in connective tissue septum. x 500

Leucine aminopeptidase zymogram of bruised muscle homogenates after isoelectric focussing at 25 watt constant power for 1.5 hours in PAG with ampholine buffer range pH 3.5-9.5. The gel was incubated in aqueous substrate medium for 30 minutes at 37°C.
Bruise 4 hours old. Negative or slightly positive non-specific esterase staining of apparently normal muscle fibres.

Bruise 24 hours old. Unchanged and increased non-specific esterase staining in necrotic muscle fibres.

Bruise 48 hours old. Strongly positive non-specific esterase staining in interstitial fibroblasts.

Esterase zymogram of bruised muscle after isoelectric focussing at 25 watts constant power for 1.5 hours in PAG with ampholine buffer range pH 3.5-9.5. The gel was incubated in aqueous substrate medium for 15 minutes at 25°C.

Esterase zymogram of bruised muscle after isoelectric focussing at 25 watts constant power for 2.5 hours in PAG with ampholine buffer range pH 4.5-6.5. The gel was incubated in aqueous substrate medium for 15 minutes at 25°C.

A representative densitometer scan of electro-focussed non-specific esterase isoenzymes obtained from bruised muscle, showing the 17 peaks represented in all the muscle samples. This particular bruise was 24 hours old. The gel had an ampholine buffer range of pH 4.5-6.5

Bruise 144 hours old. Moderate diffuse and strong punctate creatine phosphokinase staining in apparently normal muscle fibres.

Bruise 24 hours old. Unchanged creatine phosphokinase staining in severely necrotic muscle fibres.

Bruise 48 hours old. Decreased creatine phosphokinase staining in necrotic muscle fibres.

Bruise 144 hours old. Strongly positive creatine phosphokinase activity in the leucocytic exudate. The necrotic fibres show decreased activity.
3.47 Bruise 48 hours old. Strongly positive creatine phosphokinase staining of fibroblasts in connective tissue septum. x 320

3.48 Creatine phosphokinase zymogram of bruised muscle, leucocytes, plasma and erythrocytes after isoelectric focussing at 25 watt constant power for 1.5 hours in PAG with ampholine buffer range pH 3.5-9.5. The substrate was incorporated in a 2% agarose gel which was applied to the electro-focussed gel for 30 minutes at 37°C.

3.49 Creatine phosphokinase zymogram of bruised muscle, leucocytes and erythrocytes after electrophoresis at 250 volts for 90 minutes in cellulose acetate plates. The substrate was applied to a second cellulose acetate plate as a 0.5% noble agar gel. This plate was firmly applied to the first and the pair incubated for 20 minutes at 37°C.

3.50 Bruise 48 hours old. Variation in adenosine triphosphatase staining in normal muscle fibres according to fibre type in apparently normal muscle. x 50

3.51 Bruise 24 hours old. Variation of adenosine triphosphatase staining according to fibre type is maintained in areas of muscle necrosis. x 125

3.52 Bruise 24 hours old. Very mild and therefore equivocal adenosine triphosphatase staining of fibroblasts in connective tissue septum. x 500

4.1 Sequential pH measurements of normal muscle sampled 1 hour post mortem and homogenised in 5mM sodium iodoacetate. Page 145

4.2 Sequential pH measurements of normal muscle sampled 24 hours post mortem and homogenised in 5mM sodium iodoacetate. Page 145

4.3 Ranges of fore and hind limb pH values in 3 hour post mortem samples of bruises of different ages. Hind limb values are offset to the right for clarity. Page 146
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