Title - Amino acid analysis from aged beef at various locations along the M. *longissimus dorsi.*

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Background - The precise biochemical reactions which occur during the ageing period remain unclear. It is thought that endogenous enzymes and myofibrillar proteins are involved in the tenderisation process during ageing. A lot of research in meat science has focused on these two aspects. The proteolytic action of these enzymes on the myofibrillar fraction results in the production of poly-peptides which serve as substrates for the peptidyl peptidases and the aminopeptidases. Therefore increased myofibrillar protein degradation can result in elevated levels of free amino acids. We investigated the changes in these amino acids, in order to enhance our understanding of the biochemical processes occurring during *postmortem* ageing of meat.

Objectives - The present study was conducted to investigate the release of free amino acids over the ageing profile form bovine M. *longissimus dorsi* (LD). The variation in amino acid levels at eight separate sites along the LD was also analysed.

Methods - Three Hereford Cross heifers were slaughtered conventionally. The LD was excised from the carcass at 1hr *postmortem* and stored at 4°C for 15 days. At 1h, 1d, 3d and 15d *postmortem* samples of approximately 40g weight were excised from each of eight locations along the LD. Extractions of these samples, were carried out immediately after slaughter using a procedure adapted from Nakai *et al.*, (1995). Following homogenisation of twenty five grams of muscle in 50ml dH_2O, TCA (50%) was added to bring the final concentration to 5%. After centrifugation at 3,600rpm for 30mins, the supernatant was filtered through cheese cloth (X8) and stored at -20°C until analysis. Free amino acids were determined directly using 20µl of the whole supernatant. Amino acid analyses were performed with an amino acid analyser LC3000 (Eppendorf, Hamburg, Germany). Samples were applied to the ion exchange column (a spherical resin 10% DVB cross-linked polystyrol, 4X125mm) in citrate buffer at pH 2.2. Ninhydrin was used for post-column derivatisation of the amino acids. Separation was achieved with a multi-step pH and ionic strength gradient.

Results and Discussion - The average content of each individual amino acid is presented in Table 1. There were no significant variations in the amino acid content along the LD at any one time *postmortem* (Figure 1). Between two and ten fold increases were observed in many amino acids over the ageing period. Seven amino acids, which increased incrementally over the 15 days *postmortem*, were detected. These consisted of the hydrophobic amino acids, leucine (Leu) and isoleucine (Ile), the basic amino acids, histidine (His), lysine (Lys), and arginine (Arg), the aliphatic serine (Ser) and the imino acid proline (Pro). Two aliphatic aminoacids, alanine (Ala) and glycine (Gly), and the acidic glutamine (Gln) increased in a non-increment manner over the 15 day ageing period. While both β-alanine (β-Ala) and glutamic acid (Glu) increased after 1 day ageing, these concentration had returned to that of 1 hour, or lower after 15 days ageing. Feidt *et al.*, (1996) have monitored the increases in amino acid from 3 days to 15 days *postmortem*. In this period they observed increases in all detected amino acids. While most of the amino acid in this report also increased over the ageing process the extent of these increases in much greater than that reported by Feidt *et al.* (1996). Although enzymes were not monitored in this study, it is likely that the increased release of amino acids over the ageing process is due to the action of protease systems. It is established that proteolytic degradation of muscle proteins occurs over the ageing process. While there are various opinions as to what enzyme systems are involved the role of the calpains has been extensively reviewed (Koohmaraie, 1996). The action of these enzymes results in the production of substrates for other enzymes such as peptidyl- and amino- peptidases which in turn gives rise to amino acid production (Toldra et al., 1995).
The effect of the observed alterations in free amino acids, over the ageing period in muscle, on quality parameters has yet to be established.

Conclusions - Increases were observed in the levels of amino acids in bovine LD over the ageing process. No variations were noted between the free amino acids measured at each of eight locations along the length of muscle.

Literature

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Table 1. Free amino acid concentrations (μmol/ml) from bovine M. *longissimus dorsi* (LD) over the ageing process. Values represent the mean value from eight locations along the LD from each of two animals. Only amino acids which were detected at all time points are shown. Sampling was carried out at 1h, 1d, 3d and 15d *postmortem*.

	Postmortem storage time at 4°C			
	1h	1d	3d	15d
Asp	56.9	90.7	60.9	87.7
Ser	27.6	54.4	86.7	219.8
Glu	127.3	134.0	85.9	126.2
Gln	672.4	1248.4	878.2	760.7
Pro	25.6	47.2	52.0	52.71
Gly	149.3	294.1	234.4	232.9
Ala	423.7	861.7	815.5	855.0
Ile	21.0	43.5	58.7	138.9
Leu	33.8	70.2	84.8	246.1
B-ala	34.0	55.3	46.1	28.5
His	13.4	24.0	33.7	54.7
Lys	30.2	58.4	71.0	152.6
Arg	31.7	55.9	58.2	127.8

Figure 1. Total free amino acids in beef M. *longissimus dorsi* during ageing (n=3). Average values obtained at 1h,1d, 3d and 15d *postmortem* are presented. Units are μmol/ml.