Microdialysis as a technique to determine extracellular metabolites in pig carcasses

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Background

The pH development post mortem is of major importance to meat quality characteristics like colour, water-holding capacity and ultimate pH. The course of pH-decrease is determined by the metabolic state of the muscles at the time of stunning (Bendall, 1951), which is the result of a wide number of environmental factors acting on the genetic prerequisites of the animal. Stunning and killing will further change the metabolic state, due to the excessive release of Ca^{++}. This release will force the muscle to contract and imply a certain degree of membrane instability allowing for intracellular substances to leak out into the extracellular space. Consequently, the concentration of extracellular substances may indicate the level of stress to which the animals have been exposed and may be used to predict ultimate meat quality characteristics at an early stage.

The microdialysis technique has since its advent in 1966 (Bito, 1966) proven itself to be a valid method to collect extracellular substances and has been used extensively in human brain and muscle research. In this first series of experiments, we have focussed on measuring substances related to the energy metabolism of the muscles as these may influence the course of pH-decrease.

Objectives

The objectives of this investigation is to obtain basic information of extracellular concentrations of metabolites related to energy metabolism and to validate the microdialysis technique as a method to collect extracellular fluid.

Materials and methods

In all, 16 pigs were included in the investigation. These were divided within litter onto 4 individual groups, each exposed to different pre-slaughter procedures and stunning methods. One group constituted of a control, which was exposed to as little stress as possible before stunning and stunned by CO_2. A second group was exposed to a treadmill exercise bout to exhaustion and stunned electrically. The third group was given a dose of adrenalin 0.2 mg/kg live weight 15 hours prior to the experiment and stunned by CO_2, and the 4th group was exposed to the same treadmill exercise as group 2, but stunned by CO_2.

Microdialysis catheters (CMA 60, Solna, Sweden) were inserted into the longissimus dorsi 25 minutes after bleeding immediately after evisceration. Flow rate was 0.5 microliter/min and collection was started 35 minutes after bleeding and repeated at 15-minute intervals until 180 minutes after bleeding. pH and temperature were recorded and muscle biopsies (Bergstrom, 1963) were taken at the same time intervals. The carcasses were transported to a neighbouring laboratory and thus not chilled during the experimental period. Dialysate measurements included lactate, glucose, pyruvate and urea using the CMA-analyzer (Solna, Sweden), and lactate was measured on the biopsy samples using a spectrophotometric method (Passonneau and Lowry, 1993).

Results and discussion

The courses of temperature and pH declines are listed in figures 1 and 2 and indicate the variation in post mortem energy metabolism that has been obtained by the different preslaughter treatments. The adrenaline-treated pigs would develop DFD meat whereas the exercised, particularly the electrically stunned pigs are more at risk of developing PSE due to the unsuited temperature and pH conditions. The dialysis catheters used have a "cut of value" of 20 Kdalton, meaning that only relatively small molecules can pass through the membrane. Concequently, there is very little risk of further enzymatic degradation of substances in the dialysate. The glucose concentrations are given in figure 3. Adrenaline treatment seems to result in lower concentrations of extra cellular glucose whereas the other treatments do not seem to deviate much from each other. Whether the initial increase is a result of actual changes in concentrations in the extracellular space or merely a reflection of a slow-developing equilibrium is not known at the present stage. A similar initial increase is also observed in lactate concentration (fig. 4). If it reflects a slow-developing equilibrium, one of the reasons may be that the molecule is charged, which is known to influence diffusion. It was quite surprising to observe a rather constant concentration after the initial increase. This is in contrast to both a concomitant decrease in pH and an increase in lactate concentrations in biopsy samples (not shown). If it represents the true condition, one might assume that protons may diffuse more easily and that there during the experimental period are some limitations to the diffusion of lactate out of the cells. We are currently investigating this phenomenon. For pyruvate (fig. 5), a steep decrease is observed up till 80-120 minutes after bleeding depending on the preslaughter treatment. From thereon very few changes occur. In these early stages post mortem, the production of urea reflects the degradation level of nucleotides, primarily ATP. Exercised and electrically stunned animals produce more urea than the other treatments apparently (Fig. 6), which is acceptable considering the exhaustive work to which the animals were exposed combined with the forceful contractions during the actual stunning procedure.

Conclusions

The method seems to be able to detect differences caused by preslaughter treatment, which is a necessary prerequisite for any method intended to be used for early prediction of meat quality characteristic. Whether it represents true extracellular values is not important in this case. It is limited in its use to describe the dynamics of the early post mortem metabolic events, however, it can give valuable information on the exchange of substances both in the live animals during stressful situations as well as in the carcasses. This information can improve our understanding of the mechanisms underlying the development of meat quality characteristics. Further investigations are needed though to fully evaluate the potentials of this methodology in meat research.

References

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Bito, L., Davson, H., Levin, E., Murray, M., Snyder, N. (1966) The concentration of free amino acids and other electrolytes in cerebrospinal fluid, in vivo dialysate of brain, and blood plasma of the dog. *J. Neurochem, 13, 1057-1067.*

Passonneau, J. V., Lowry, O. H. (1993). *Enzymatic analysis : A practical guide.* The Humana Press Inc. New Jersey, USA.

Legends to tables

Figure 1. Temperature decline in Longissimus Dorsi in the four groups of pigs
Figure 2. pH-decrease in Longissimus Dorsi in the four groups of pigs
Figure 3. Concentration of pyruvate in dialysates sampled during 3 hours post mortem
Figure 4. Concentration of glucose in dialysates sampled during 3 hours post mortem
Figure 5. Concentration of lactate in dialysates sampled during 3 hours post mortem
Figure 6. Concentration of urea in dialysates sampled during 3 hours post mortem

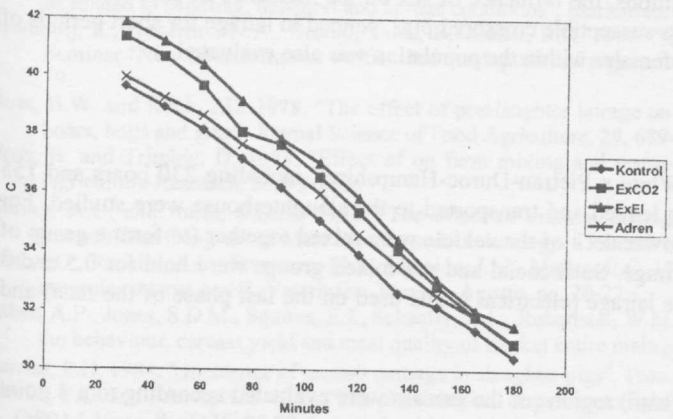

Figure 1

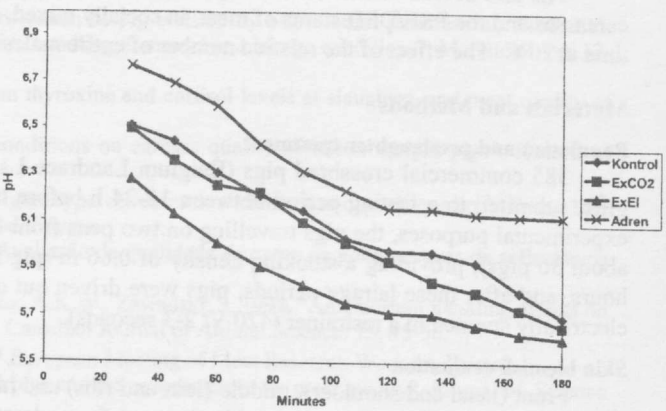

Figure 2

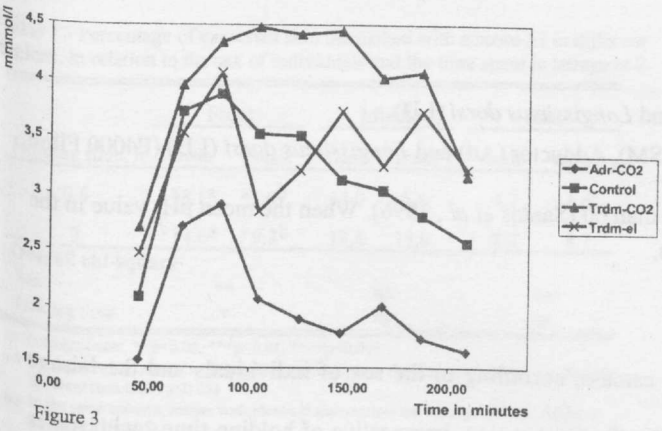

Figure 3

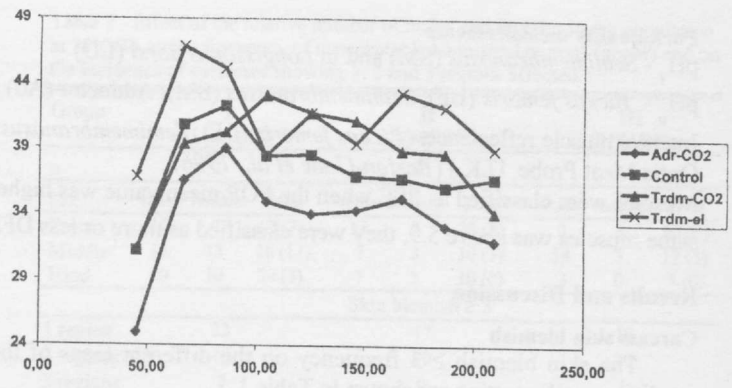

Figure 4

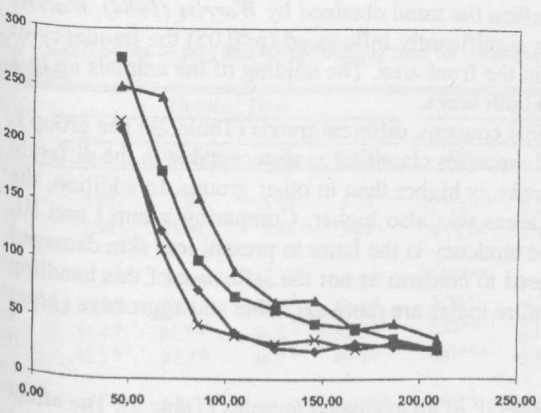

Figure 5

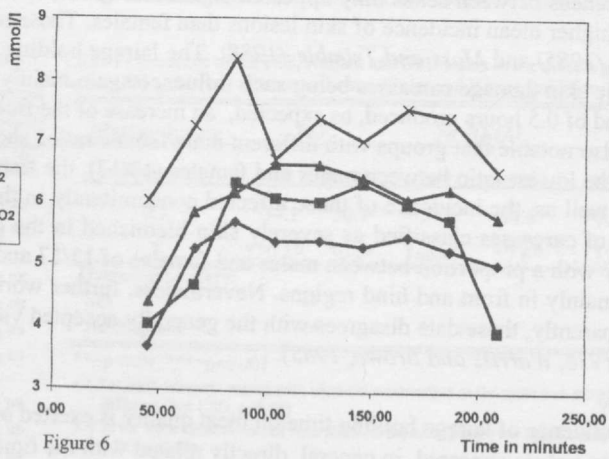

Figure 6