DEVELOPMENT OF AN ULTRA-FILTRATION TECHNIQUES FOR CONTINUOUSLY MONITORING BLOOD GLUCOSE AND LACTATE IN BROILER CHICKENS.

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Background
In poultry the welfare ante mortem and the quality of the carcass meat post mortem are easily adversely affected by catching and handling methods on the farm, during transport and at the slaughterhouse (Figure 1). Consumers are presently demanding better conditions for animals and improved quality of products in the whole production chain. The pre-slaughter handling of the animals is increasingly a matter of debate.

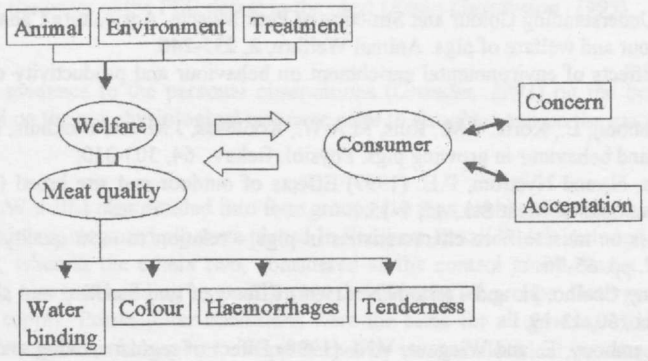

Figure 1: Transport factors that affect welfare and meat quality.

Several factors cause stress during transport, such as separation from a familiar environment, the process of catching and crating, transport, changes in temperature, ventilation etc. Climatic conditions, loading density, duration of transport, cold draughts, heat stress, social stress, vibrations, restraint and noise have adverse effects on the welfare of the animals unaccustomed to it (Knowles & Broom, 1990; Mitchel & Kettlewell, 1998). Indicators of poor welfare include behavioural, physiological, and immunological responses indicating coping difficulties, injuries and effects on the carcass meat of slaughtered animals and effects on the health status of animals, which are not immediately slaughtered.
It is assumed that stress before slaughter leads to an increased breakdown of glycogen and glucose and an increased production of lactate. In broiler chickens stress results in pale, soft and exudative (PSE) meat. In addition, the physiological response to stressors from the environment is partly influenced by the genotype of the animal (Nicol & Scott, 1990). Nevertheless, the occurrence of dark, firm and dry (DFD) meat is more readily attributed to effects of the transport environment and is less variable amongst genetic lines. When the animal is fatigued and the energy store is exhausted, no acidification and dark coloured meat will occur (Hillebrand, 1993). Monitoring physiological processes in blood or other body fluids is possible by frequently taking samples for off line analysis. Such sampling techniques are often physically or psychologically stressful, or pose a severe restraint on the circumstances under which sampling can be done. For small animals repeated sampling can imply a compromise of their physiological capacity to compensate for the loss. Even if samples can be taken stress-free, animals must often be housed individually in an experimental environment that allows easy sampling.

Objective
Recently we have developed an ultra-filtration collection probe for the sampling of blood or (subcutaneous) tissue fluid that may be used for continuous, stress-free sampling in a naturalistic environment.

Methods and techniques
The ultra-filtration system is based on inserting into the relevant body compartment a semi-permeable membrane, which is connected to a collection tubing that stores the fluid sample. Its small inner diameter (125 µm) prevents diffusion. A low, continuous sampling flow (50 nl/min.) is driven by a pulse-free vacuum pump (Figure 2) (Moscone et al., 1996). As only tissue fluid is stored, the ultra-filtrate always has 100% recovery. The membrane (cut-off 20 kD) keeps out degrading enzymes (components). The entire system is small and may be attached to the animal. Afterwards the ultra-filtration-collection probe is removed and the contents are analysed in 20 nl fractions using a bi-enzyme reactor (glucose oxidase/horseradish peroxidase or lactate oxidase/horseradish peroxidase, using a ferrocene buffer as mediator) with electrochemical detection in a flow injection system (Elekes et al., 1995).
We have applied for the first time this technique to sample blood ultra-filtrate for 8 hours in free moving, group housed chickens to monitor glucose and lactate levels. A membrane was surgically inserted into the right wing vein. The collection tubing and pump

were attached under the wing in a plastic bag. After recovery the birds were returned to their pens. The probe did not seem to hinder the chickens, or attract unwanted attention from other chickens.
At 3 days after surgery the pump was started, and the probe ran for 8 hours during which the birds were crated and transported for 1,5 hours. Afterwards the probe was removed and the collection tubing separated sealed and stored at 4°C until analysis. The sample was analysed to give a 5-minute resolution profile of glucose and lactate (Figure 3).

Preliminary results and discussion

We have explored this technique to sample blood ultra-filtrate for 8 hours in freely moving, group housed broiler chickens to monitor glucose and lactate levels. Analysis was done to provide 5-minute resolution profiles of glucose and lactate (Figure 3). Smooth profiles were obtained in about 50% of the analysed collection tubings. The other tubings were severely obstructed with air bubbles that were created by evaporation and absorption of fluid by the collection tubing itself. Air is compressible and disturbs the linearity of the analysis in time, thereby making it impossible to place a proper time axis to the part of the profile that could be analysed. Also, in the undisturbed profiles unusually high concentrations of glucose and lactate were found. Therefore, expression the data as ratio lactate/glucose may provide a more accurate index for metabolic activity. Preliminary tests showed that using different materials (e.g. fused silica tubing) for storage tubing might solve these problems.

Comments and conclusion

We describe here an ultra-filtration collection device that is portable, even for small experimental animals, allows continuous stress-free sampling, has a long term sample storage, and a high resolution profiling capacity. So, the device may become an excellent tool for monitoring various physiological compounds in situations where blood or tissue sampling is difficult or impossible. The ultra-filtration collection probe is an aspecific sampling system. It may be adjusted for sampling in other species or different tissues, or analyse for different components. Other membranes, sample stability and small volume analyses will have to be studied. Thus far the profiles obtained with the ultra-filtration collection probe have not yet been compared to values in whole blood within subjects, and such validation is necessary to further develop the probe for use under practical conditions. The system can be used to monitor animal welfare and judgement of quality in broiler chickens and other meat animals.

References

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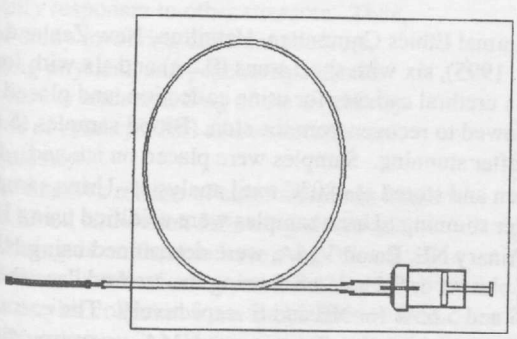

Figure 2: Schematic drawing of the ultra-filtration collection probe. A: 4 cm semi-permeable membrane; B: 1 cm fused silica connection tubing; C: nylon blend collection tubing, 30 cm per hour sampling time at a flow of 50 nl/min.; D: air collector; E: restriction tubing, 8 cm fused silica tubing of 15 µm inner diameter; F: under-pressure pump, 1.2 ml monovette, 6250 Pa pressure; G: plastic bag to be attached to the wing of the chicken, parts outside this area are inserted into the wing vein.

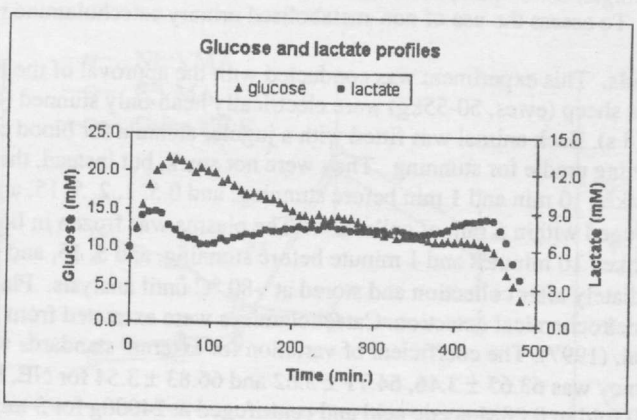

Figure 3: A 5-minute resolution profile of glucose and lactate