European Journal of Applied Sciences – Vol. 12, No. 06
Publication Date: December 25, 2024
DOI:10.14738/aivp.1206.17823.
Swatland, H. J. (2024). A Review of Abattoir Reflexes in Relation to Anaerobic Glycolysis and Meat Quality. European Journal of Applied Sciences, Vol - 12(06). 19-28.
SERVICES FOR SCIENCE AND EDUCATION

A Review of Abattoir Reflexes in Relation to Anaerobic Glycolysis and Meat Quality

H.J. Swatland
Department of Animal Biosciences, University of Guelph

ABSTRACT

Current research on meat quality is dominated by correlative studies of animal genotypes and histochemical fiber types, correlating features of live animals with features of commercial importance in their meat. But between live muscle and meat, there is an epigenetic realm where random factors mediated by animal treatments and their nervous systems may have strong effects. They have the potential to obscure real correlations between live animals and meat quality, or to produce spurious correlations. Physiological studies in abattoirs may prove things one way or the other.

Keywords: Electroencephalography, Electrocorticography, Electromyography, Meat quality.

INTRODUCTION

A reasonable place to start for this historical review of the electrophysiology of meat is with the discoveries of Luigi Galvani (1737-1798). Frog legs might be excluded from the definition of meat by some authorities, but as striated skeletal muscle being prepared in a kitchen for human consumption, this may be too restrictive and missing some great science. Galvani was intrigued by the post mortem kicks of isolated frog legs hanging on metal rails in a kitchen. This started discoveries in bioelectricity, the physics of electricity, and the literary fiction of Mary Shelley (Frankenstein).

Meat science as we know it today, started in the 1930s with biochemists trying to understand how a contractile and extensible tissue like striated skeletal muscle could undergo rigor mortis to become a non contractile and almost inextensible food commodity – meat. Their discoveries had an amazing scientific future. They discovered how calcium ions control muscle contraction – and confirmed many of the key factors in our contemporary explanations of muscle contraction – actin, myosin, sarcoplasmic reticulum, tropomyosin, troponin, and sliding filaments [1, 2]

In the early days, an important adjunct to biochemistry was the physiological testing of skeletal muscles as they became inextensible during the development of rigor mortis [3, 4]. The rigorometer was developed, new at the time for food scientists, but drawing on a century of research by muscle physiologists [5]. However, this was a real paradigm shift. Physiologists require muscle strips with slow post mortem metabolism so that muscle contraction may be measured as a response to direct or neural excitation, whereas rigorometers measure the loss of extensibility when a muscle strip going into rigor mortis is loaded. This requires a supply of muscle immediately post mortem – many experiments

started with rabbit muscle, and then moved on to beef from a nearby abattoir. This gets us to the main objective of this review. The pioneers in this field certainly recognized that an excised sample of skeletal muscle might be affected by the damage caused by excision – transected myofibre membranes, denervation, a change in temperature, gaseous environment and a temporary change in length. Not withstanding these problems, excised muscle strips laid the ground work for all our contemporary understanding of meat science sarcomere length, cold shortening, etc. But what happens to a strip of muscle that remains in the carcass without being excised?

EXCISION AND MEMBRANE RECOVERY

The main concern with using excised muscle strips to understand events in an almost intact meat carcass is the problem of cutting through muscle fibre membranes at each end of the strip. From first principles of membrane physiology, one might expect the short circuiting of resting potentials across membranes followed by uncontrolled action potentials along muscle fibres to invalidate any further physiological measurements. Luckily for meat science, this did not happen and excised muscle strips became the foundation for much of what we now believe, but how? The answer came from work investigating the movement of extracellular space markers into transected muscle fibers [6]. After a while, the plasma membrane repairs the cut end, sufficient to allow membrane pumps to restore resting potentials.

A profound question relative to abattoir reflexes is how long after exsanguination do neuromuscular pathways survive (taking the termination of blood flow from the puncture of the *anterior vena cava* as time zero). In pigs, it all depends on where the stimulatory electrodes are placed and on where the resulting muscular contractions are evaluated, but from 13 to 18 minutes gives the order of magnitude [7]. The profundity of this question is that it relates both to our understanding of the science of humane slaughter of meat animals and abattoir reflexes affecting meat quality. Humane slaughter cannot be included in this review – it is difficult topic to approach scientifically, just like the polemics of animals rights activists versus ritual slaughter lobbies. Even correlating reflex activity with meat quality is difficult. Reflex activity affects anaerobic glycolysis, and pH affects meat quality, but where are the studies of a direct correlation of reflex activity with meat quality?

Using electromyography to explore how neural excitation might affect postmortem muscle metabolism was developed by Schmidt et al. [7]. Some breeds of pigs at the time were producing pale, soft, exudative (PSE) pork and it was reasonable to suspect that this might be caused by postmortem neural excitation causing muscle activation with a commensurate increase in post mortem glycolysis. After many years it was found that the increase in post mortem glycolysis causing PSE was a result of the release of calcium ions from the sarcoplasmic reticulum [8]. However, a start had been made on a much wider scope of investigation as yet unfinished– how do abattoir reflexes affect postmortem glycolysis and meat quality?

The starting experiments in this field were by Bendall [9], working with pigs and pH-dependent aspects of pork quality. Still seeking an explanation for PSE pork, he showed that injection of curare to block neuromuscular junctions produced a major reduction in post mortem glycolysis. This did not explain the urgent problem of PSE pork from pigs with a

genetic alteration of calcium release channels in the sarcoplasmic reticulum, but it is the basis for understanding how abattoir reflexes affect meat quality, especially in pork, and who knows how many other animals.

An experiment to look at the natural EMG activity in the porcine *vastus lateralis* muscle on the non-shackled side (isotonic muscle contraction not prevented by shackling) is shown in Fig. 1. Here the kicking of the free limb is seen (Fig. 1, A). A stimulatory electrode inserted near the lumbar spinal cord (1 v square wave, 32 msec duration at 1 Hz) then caused an EMG response and contraction for several minutes until it declined (Fig 1, C), but could then reactivated (Fig. 1, D) by increasing the voltage to 100 V until the response diminished (Fig. 1, E) and the EMG electrode picked up the distant constant stimulatory voltage (Fig. 1, F). This showed that reflex kicking in an unshackled limb was terminated by the central nervous system, not by an inoperative peripheral nerve [10].

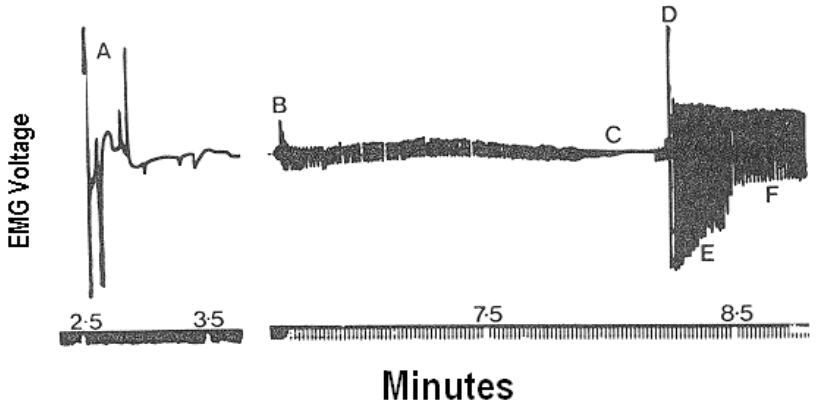

Fig. 1: EMG activity of free kicking in the *vastus lateralis* of a pig carcass (minutes postmortem).

Shackling a newly slaughtered pig by one hind limb causes a massive neurological response possibly involving brain stem reflexes, Golgi tendon organs and neuromuscular spindles in the free limb. Before shackling (time 0 in Fig. 2) there was diminishing EMG activity (as in Fig. 1, A), now followed by bursts of kicking (Fig. 2, B and C), then slowly diminishing activity (Fig. 2, D).

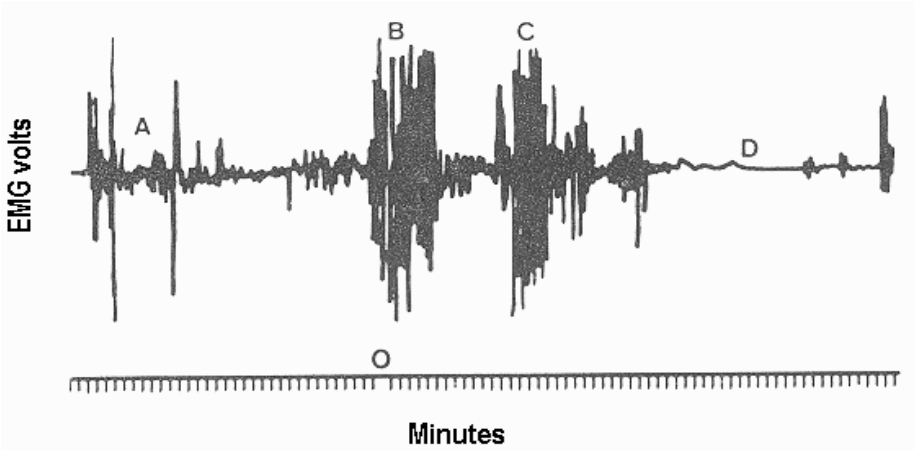

Fig. 2: Electromyography of isotonic reflex activity in the free limb of a pork carcass following shackling at time 0.

Does a rapid decline in pH caused by anaerobic glycolysis reduce the response of a muscle to further endogenous stimulation [10] as it does to exogenous stimulation by electrodes [11]? EMG activity declines rapidly in stress-susceptible pigs [12]. But where do post mortem muscle contractions and EMG activity originate?

Two methods are available in an abattoir to detect brain activity in a slaughtered animal; an electroencephalogram (EEG) detects electrical activity from electrodes placed on the skin of the head, while electrocorticography (ECoG) has electrodes on the surface of the brain. Pigs have only a small motor cortex [14]. But even with the difficulty of placing ECoG electrodes near the motor cortex of a pig, it is possible to detect signals that completely match movements of an unshackled limb [15]. However, might the signals in Fig.3 be a sensory response to the reflex kicking of the limb originating from elsewhere?

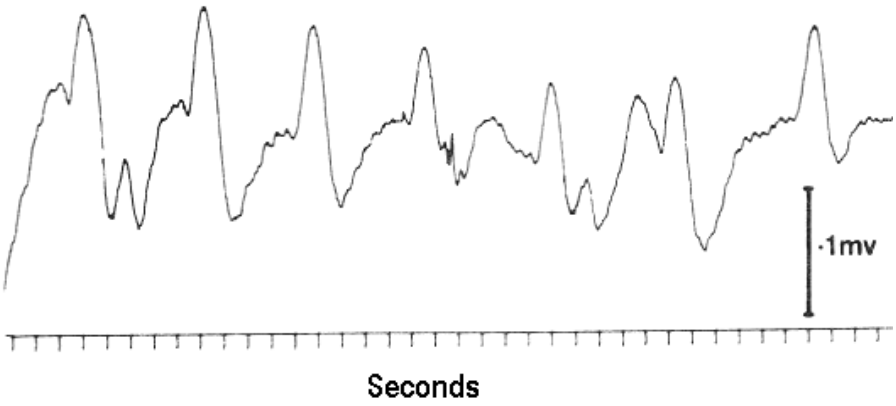

Fig. 3: Electrocorticographic activity from the brain of an electrically stunned pig matched to observable kicking of an unshackled hind limb [15].

An attempt to answer this question was to surgically implant electrodes in anaesthetized pigs, let them recover from the procedure for many days, then slaughter them after electrical stunning. All precautions were taken under veterinary supervision to avoid any distress to the pigs. Electrical stunning is widely used for pigs in an abattoir and there are many research papers recommending voltages and times to be applied with electrodes of varying design and electrical continuity across the head of a pig [16], but ECoG electrodes reveal the voltages that remain after penetrating the high resistance of skin and skull bone. Some complex apparatus is required to make physiological measurements in the difficult working conditions of an abattoir (Fig. 4). The main difficulties are to protect the apparatus from water, mechanical damage and static electricity (which is surprisingly high in such a wet environment).

All this instrumentation did little to identify the source of abattoir reflexes in pigs, although it did provide some important information about the physiology of slaughtering.

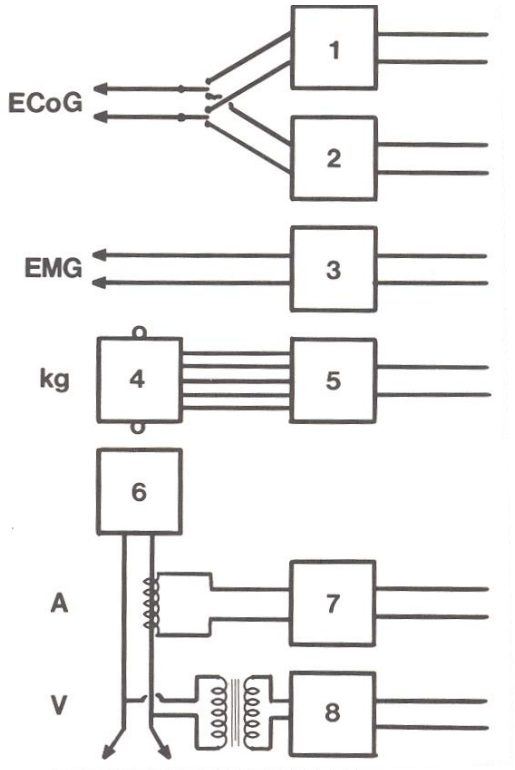

Fig. 4: Apparatus for measuring ECoG, EMG, kilograms carcass weight in the shackling chain and stunning current amps and volts. A switch was required to separate the alternating current of the stunner (1) then to change to the ECoG voltage (2). A high gain amplifier was required to for the EMG signal (3). A signal conditioner was required to provide an analog voltage from the load cell (4) in the shacking chain recording reflex activity (5). Circuitry was required to obtain both the stunner amperage and voltage (6, 7 and 8) [17].

Fig. 5 shows some ECoG data from the cerebral cortex of a resting pig after recovering from the surgery.

Despite attempts to implant electrodes above the small motor cortex of this pig, the responses were all sensory. Perhaps sensory responses spread widely on the cerebrum in pigs? Who knows? It is difficult to find any new research papers on this topic. One of the reflexes commonly observed in slaughtering pigs is a head down choking reflex, as seen in a family dog that has just eaten something to large with a threat to blocking the trachea.

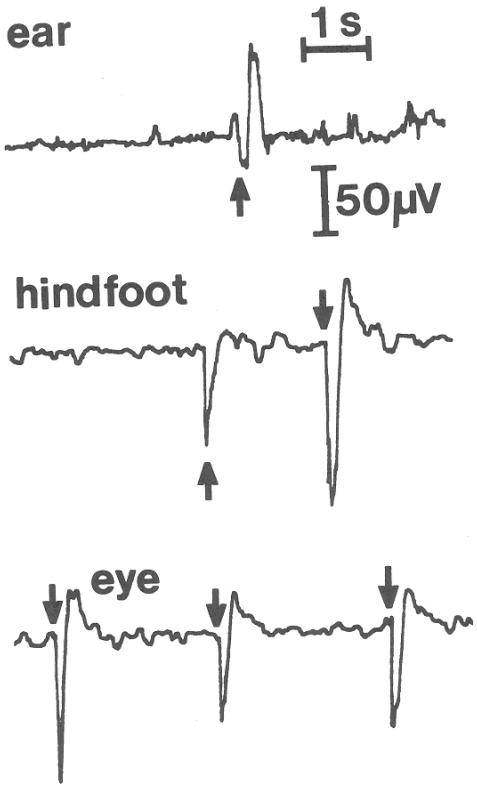

Fig. 5: ECoG recordings from a resting pig after recovery from the surgical implantation of electrodes. The arrows show when touching various parts of the body.

Fig. 6 show this does not originate from near the motor cortex, despite corresponding EMG activity for the muscles involved. Perhaps this is a brain stem reflex?

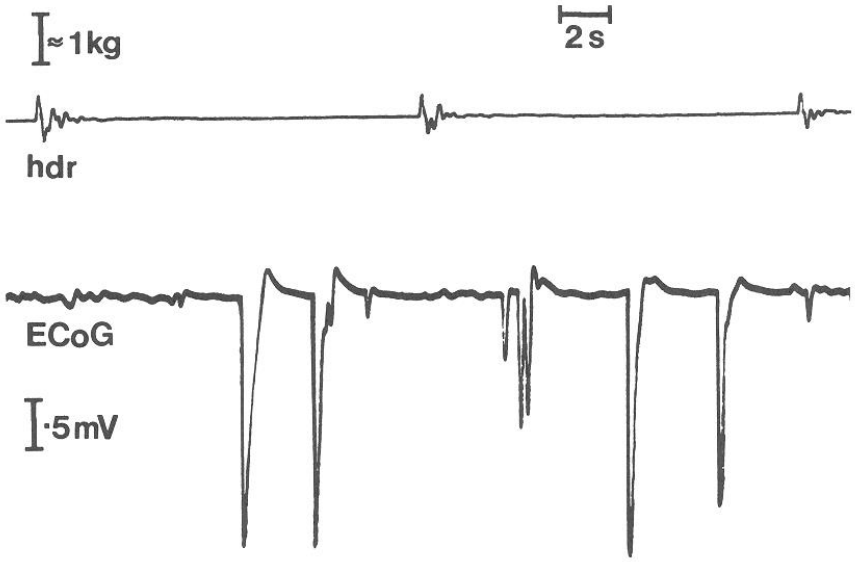

Fig.6: Head down reflexes (hdr) detected in a shackling chain with no observable relationship to ECoG activity near the motor cortex.

Despite not finding where the head down reflex originated, the apparatus revealed some new information about how stunning currents for pigs actually work. Fig. 7 shows the externally applied stunning current to a pig. And Fig. 8 shows what reached the cerebral cortex. The main point to note is that whatever the voltage applied to the outside of pig skull, the operative stunning current across the brain is really quite small.

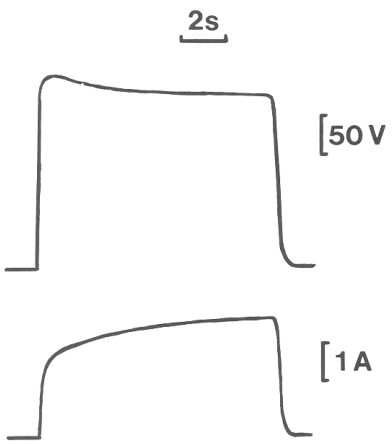

Fig. 7: The externally applied stunning current to a pig.

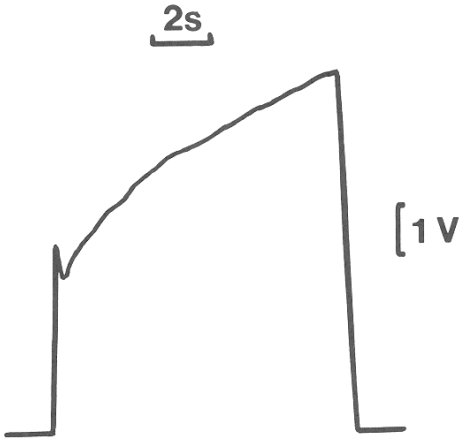

Fig. 8: How the stunning current in Fig. 7 arrived on the cerebrum.

If post mortem reflex activity has any effect on pH dependent aspects of meat, then the obvious place to look is at differences between the isometric contraction of a shackled hind limb and the isotonic contraction of the unshackled limb. Fortunately, it had just been discovered that electrical capacitance could be used to follow anaerobic glycolysis and pH decline. The basic work on this topic was undertaken in the 1930s using electrical resistance with a direct current, but resistance is temperature dependent whereas an alternating current measures electrical impedance, a combination of resistance and capacitance. To cut a long story short, electrical capacitance, almost independent of carcass temperature, is a convenient way to assess to anaerobic glycolysis from measurements on exposed muscle of a pork carcass. Essentially, measuring the muscle fibre membranes that have lost their dielectric properties post mortem is a way to detect post mortem glycolysis. There are differences between left and right sides of a pork carcass – the unshackled side with isometric contraction advances faster than the shackled side with isotonic contraction. Thus, abattoir reflexes do have an effect on pH and pork quality [18].

ANAEROBIC GLYCOLYSIS

Finding relationships of abattoir reflexes with meat quality in pork, led to investigating the situation in beef. Whereas PSE and reflex contractions were the main focus for pork, the main focus for beef was external electrical stimulation which, in the 1950s, attracted great interest as a method to accelerate the development of rigor mortis, thus enabling early meat cutting [19]. The link between reflex activity and anaerobic glycolysis and pH decline was that excised bovine muscles often twitch (Fig. 9). Finding EMG spikes from individual muscle fibres interspersed with smooth motor contractions prompted a paradigm shift in understanding anaerobic glycolysis. In the classic rigorometer studies of post mortem metabolism [4], muscle samples were homogenized in a fluid using isotonic KCl and

iodoacetate to arrest glycolysis to preserve and prevent further *p*H changes. This averaged all the responses of individual muscle fibres, whereas there was now evidence, as in Fig. 9, of individual muscle fibres acting individually. This was analyzed histochemically using the periodic-acid Schiff reaction for glycogen. All fibres started loaded with glycogen, but microscope sections after the termination of twitching revealed those fibres that had depleted their glycogen (Fig. 10).

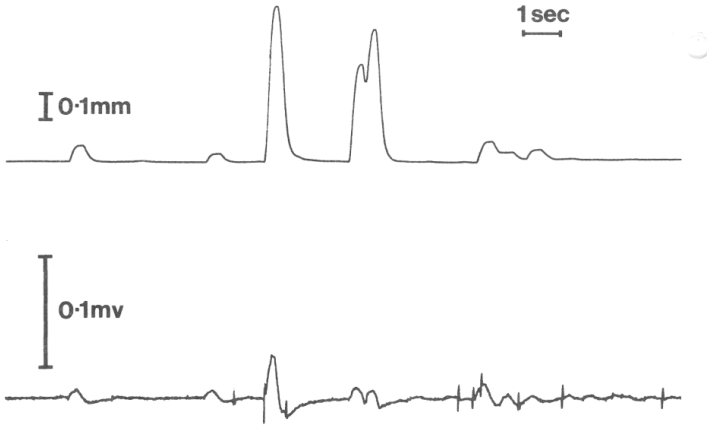

Fig. 9: Post mortem isotonic contractions (top line, 50 g load) in a strip of bovine *sternomandibularis* showing EMG activity (bottom line) with spikes from individual myofibres [20].

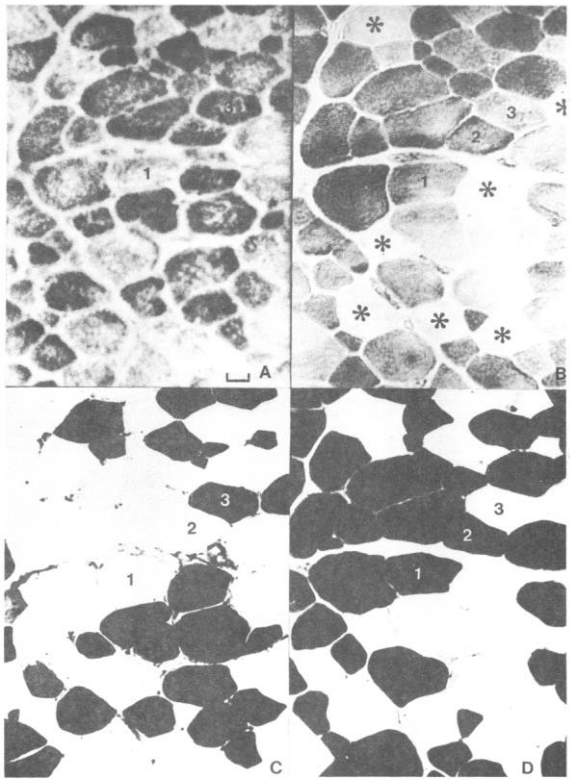

Fig. 10: Serial sections from a strip of bovine *sternomandibularis* after the completion of muscle twitching (bar scale = 20 µm). Section A shows glycogen stained by the periodic acid – Schiff

reaction, section B shows aerobic fibres with a high mitochondrial content, section C shows slow fibres with acid-stable ATPase, and section D shows fast fibres with acid-labile ATPase. The asterisks show the fast fibres that had depleted their glycogen [20].

WHAT HAPPENED NEXT?

This whole topic is now almost forgotten except for animal welfare considerations [21]. Meat science went on to empirical correlations of animal genetics and muscle fibre types with meat quality, ignoring the epigenetic realm where these factors interact. There were technological advances using fibre optics and diode arrays to monitor post mortem metabolism, just like the empiricism of current research using image analysis. But correlations are only the first guide to experiments proving causality, which are still needed to advance meat science.

References

[1] Marsh, B.B. (1951). A factor modifying muscle fibre synaeresis. Nature 167(4261): 1065-6.

[2] Szent-Györgyi, A. (2004). The early history of the biochemistry of muscle contraction. Journal of General Physiology, 123: 631-641.

[3] Bate-Smith, E.C. and Bendall, J.R. (1949). Factors determining the time course of rigor mortis. Journal of Physiology. 110: 47-65.

[4] Bendall, J.R. (1972). Postmortem changes in muscle. In, The Structure and Function of Muscle, Vol. 2, Part 2. 344- 309.

[5] Rosenthal, I. (1881). General Physiology of Muscles and Nerves. International Scientific Series, XXXII, Appleton, New York.

[6] Echeverria, O.M., Vázquez-Nin, G.H. and Ninomya, J.G. (1983). Structural alterations and changes in the distribution of markers in the transected skeletal muscle fibers of the guinea pig. Acta Anatomica, 116(4): 358-365.

[7] Swatland, H.J. (1975). Survival times of neuromuscular pathways in slaughtered pigs. Canadian Institute of Food Science and Technology. 8:122-123.

[8] Fujii, J., Otsu, K., Zorzato, F., Deleon, S., Khanna, V.K., Weiller, J.E., O'Brien, P., and MacLennan, D.H. (1991). Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science, Washington, 253: 448-451.

[9] Bendall, J.R. (1966). The effect of pretreatment of pigs with curare on the post-mortem rate o pH fall and the onset of rigor mortis. Journal of the Science of Food and Agriculture 17: 333-338.

[10] Swatland, H.J. (1976). Electromyography of electrically stunned pigs and excised bovine muscle. Journal of Animal Science 42: 838-844.

[11] Hammelman, J.E., Bowker, B., Grant, A.L., Forrest, J.C., Schinckel. A.P. and Gerrard, D.E. (2003). Early postmortem electrical stimulation simulates PSE pork development. Meat Science 63: 69-77.

[12] Forrest, J.D., Judge, M.D., Sink, J.D., Hoekstra, W.G., and Briskey, E.J. (1966). Prediction of the time course of rigor mortis through response of muscle tissue to electrical stimulation. Journal of Food Science, 31: 13

[13] Schmidt, G. R., Goldspink, G., Roberts, T., Kastenschmidt, L.L. and Briskey, E.J. (1972). Electromyography and resting membrane potential in longissimus muscle of stress-susceptible and stress-resistant pigs. Journal of Animal Science 34: 379-383.

[14] Breazile, J.E., Swafford, B.C. and Thompson, W.D. (1966). A study of the motor cortex of the domestic pig. American Journal of Veterinary Research 27: 1369-1373.

[15] Swatland, H.J. (1976). An electrocorticographic study of necrobiosis in the brains of electrically stunned and exsanguinated pigs. Journal of Animal Science 43: 577-582.

[16] van der Wal, P.G. (1978). Chemical and physiological aspects of pig stunning in relation to meat quality. Meat Science 2: 19-30.

[17] Swatland, H.J., Brogna, R.J. and Lutte, G.H. (1985). Electrical activity in the cerebral hemispheres of electrically stunned pigs. Journal of Animal Science 58: 68-74.

[18] Swatland, H.J. (1982). Evaluation of pH–related aspects of pork quality by capacitance measurements in an abattoir. Canadian Journal of Animal Science, 62: 725-730.

[19] Adeyemi, K.D. and Sazali, A.Q. (2014). Efficacy of carcass electrical stimulation in meat quality enhancement. Asian-Australasian Journal of Animal Sciences, 27: 447-456.

[20] Swatland, H.J. (1976). Motor unit activity in excised prerigor beef muscle. Canadian Institute of Food Science and Technology 9: 177-181.

[21] Vecerek, V., Kamenik, J., Voslarova. E., Vecerkova, L., Machovcova, Z., Volfova, M. and Konvalinova, J. (2020). The occurrence of reflexes and reactions in cattle following stunning with a captive bolt at the abattoir. Animal Science Journal. Jan-Dec 91.1. doi 10.1111/asj. 13373.