

# PRESLAUGHTER HANDLING OF PIGS AND THE EFFECT ON HEART RATE, MEAT QUALITY, AND SR-CA2+ TRANSPORT

Küchenmeister, U., Kuhn, G., Ender, K.

Research Institute for the Biology of Farm Animals, Research Unit Muscle Biology and Growth, Wilhelm-Stahl-Allee 2, D-18196 Dummerstorf

### Background

Preslaughter stress is generally thought to be of influence on meat quality parameters, mostly with a negative effect. However, the results of published experiments are not unequivocal. Genotype, transportation, lairage time, season of the year, environmental conditions and many other factors are also of effect on meat quality. One problem is to evaluate the effective level of stress on the animal. Heart rate can be used as one indication.

Although tenderness is mostly taken into account in beef investigations, it is also an essential parameter for pig meat quality. Tenderness of meat develops by the proteolytic action of the calpain/calpastatin system on different interfilament proteins, loosening the myofibrillar structure. Calpain is activated by  $Ca^{2+}$ , indicating the role of the intracellular  $Ca^{2+}$  concentration on the tenderness. The effect of  $Ca^{2+}$  is manifested by several investigations injecting  $Ca^{2+}$  (Kerth et al, 1995), marinating meat in  $Ca^{2+}$  containing solutions (Young et al., 1995) or inducing  $Ca^{2+}$  release by high pressure (Okamoto et al., 1995). Generally, a disturbed regulation of the intracellular  $Ca^{2+}$  concentration meat quality parameters pH, drip, and colour, but on the other hand an increased  $Ca^{2+}$  concentration may activate the protease calpain, tenderising the meat. So, it seems worthwhile to investigate the relationships between  $Ca^{2+}$  transport, meat quality and tenderness.

# Objectives

The objective of this study was to elucidate the impact of different kinds of stress (nose snare, electrical goad, control) just before slaughter on the development of meat quality. Especially the effect of the SR-Ca<sup>2+</sup> transport in post mortem muscle samples on the development of tenderness was of interest. To estimate the reaction of the used kinds of stress on the pigs, the heart rate was determined.

### Materials and methods

### Animals and stress

Thirty female German Landrace pigs were used. The pigs were produced and raised in single boxes in an experimental unit of the institute up to a live weight of 105 - 115 kg. The animals of this experimental unit are free of the mutation of the calcium release channel (CRC).

The evening before slaughter the three heaviest pigs were transported to the slaughter facility of the institute. The heart rate was measured for about half an hour before slaughter by using a Polar Heart Rate Monitor.

About 5 min before the slaughter one pig was gently driven into the stunning pen and stunned electrically. The second pig was also driven into the stunning box but received an additional stress by the application of a nose snare for 5 min. The third pig was stressed for five minutes before slaughter by using an electrical goad according to D'Souza et al. (1999). Immediately following the stress just before stunning a biopsy (shot biopsy device) was taken. The stunning and the following procedures were identical for all pigs. Immediately following exsanguination a muscle sample (0 h sample) of the *longissimus* muscle between the  $13^{th}$  and  $15^{th}$  rib was removed by shot biopsy. Also, at 45 min p.m. and at 4 h p.m. samples were taken.

# *Sample preparation and Ca*<sup>2+</sup> *uptake determination*

About 0.5 g of muscle tissue were homogenised and the  $Ca^{2+}$  uptake of the muscle homogenate was determined as described by Küchenmeister (1999a). A part of the homogenate was stored at -70° C and later used for protein determination and different biochemical investigations. The calcium release channel was manipulated as described earlier (Küchenmeister et al., 1999a).



#### *Meat quality measurements*

Meat quality parameters of the *longissimus* muscle were measured by standard procedures (Küchenmeister et al., 1999b). The R-value as an indication for p.m. energy metabolism was determined on 45 min samples (Honickel et al., 1977). For determination of tenderness, two slices of *longissimus* muscle (13th-15th rib) were sampled 24 h p.m. One slice was used for tenderness determination on this sampling day and the second slice was stored at about 5° C for 6 days and then the tenderness was determined using a Texture Analyser with Warner-Bratzler accessories.

### **Results and discussion**

The effect of the applied stress on the pigs is not easy to evaluate. One parameter to indicate the stress is the heart rate (HR). Figure 1 shows that the HR of the control animals (no additional stress) increased slowly in the course of the gentle movement (start about 5 min before slaughter) from the lairage box to the stunning from about 100 beats per minute (bpm) to 175 bpm. The nose snare stress started 5 min before slaughter after gently moving the pig into the stunning pan (with increasing heart rate comparable to the control animals). So, the heart rate started at about 175 bpm (comparable to Control). Surprisingly, however, the heart rate decreased in the time course of snare use down to 100 bpm, although the pigs were shrieking and pulling. This phenomenon is in agreement with earlier investigations (Geverink et al., 2002), but a plausible explanation is missing. The use of the electrical goad not only increased the HR in a short time interval up to 200 bpm, but also the pigs were running to avoid the electrical shock. So, this seems to be an effective stress.

These different levels of stress are reflected on the meat quality parameters (Tab. 1). There were no significant differences between control and nose snare pigs. However, the use of the electrical goad resulted in significant lower pH45 values and a brighter colour. Also, the drip loss and conductivity 24 h p.m. were highest in the goad group. Figure 2 shows the pH in the time course p.m. Four h p.m. the pH values were almost identical. However, the use of the goad resulted in lower values already immediately after slaughter and 45 min p.m., indicating an increased energy consumption and glycolysis p.m. by the application of the goad. This is verified by a higher R-value (IMP/ATP) 45 min. p.m.

The  $Ca^{2+}$  uptake of the sarcoplasmic reticulum (SR) of the homogenate (Tab. 2) shows generally lower values in the goad group, although not always significantly different from the other groups. The uptake 45 min p.m. was significantly lower following goad stress (independent of the state of the calcium release channel: basic or closed), but there were no differences between control and nose snare. As expected, the uptake decreased in the time course p.m. The rate of decrease was higher with basic CRC, compared to closed CRC. While the level of decrease with closed CRC indicates deterioration of SR-ATPase and SR membranes, the rate with basic CRC implies an opening effect on the CRC. A very fast and significant decrease of uptake between biopsy and 0 h samples with basic CRC was the result of applying goad stress. A reduced SR Ca<sup>2+</sup> transport is supposed to increase the intracellular Ca<sup>2+</sup> concentration compared to undisturbed Ca<sup>2+</sup> regulation.

Our hypothesis, that a reduced  $Ca^{2+}$  uptake will be of effect on the tenderness by activation of calpains because of a higher intracellular  $Ca^{2+}$  concentration could not be verified. The tenderness, determined at 24 h p.m. samples, was not different between experimental groups (Fig. 3). This also applies for samples stored for six days before tenderness measurement. The shear values were significantly reduced after the storage of the loin chops compared to the 24 h p.m. measurements, however, the increase in tenderness was not influenced by the kind of stress. The rate of glycolysis affects the extend of the tenderisation at least in beef. A low glycolytic rate p.m. results in high shear force, whereas a high glycolytic rate leads to lower shear values (O'Halloran et al., 1997), probably related to lower or higher intracellular  $Ca^{2+}$  concentrations, respectively.

### Conclusions

The immobilisation by a nose snare seems to have a very limited stress effect, indicated by decreasing HR and no significant effects on meat quality and  $Ca^{2+}$  transport. The use of an electrical goad had a major effect on all measured parameters: Heart rate,  $Ca^{2+}$  transport, meat quality, except tenderness. A reduced  $Ca^{2+}$  transport, induced by the high goad stress resulted as expected in inferior meat quality.



However, the hypothesis, that a reduced  $Ca^{2+}$  transport implies a higher intracellular  $Ca^{2+}$  concentration resulting in more tender meat could not be verified. Altogether, a stress before slaughter has to have a high level to be of significant influence on meat quality.

### References

D'Souza, D.N.; Warner, R.D.; Dunshea, F.R.; Leury, B.J.: Comparison of different magnesium supplements on pork quality. Meat Science 51 (1999), 221-225

Geverink, N.A.; Schouten, W.G.P.; Gort, G.; Wiegant, V.M.: Individual differences in behavioural and physiological responses to restraint stress in pigs. Physiology & Behavior 77 (2002), 451-457

Honikel, K.O., Fischer, C.: Eine Schnellmethode zur Bestimmung von PSE- und DFD-Fleisch beim Schwein. Fleischwirtschaft 5 (1977), 1015-1019

Küchenmeister, U.; Kuhn, G.; Wegner, J.; Nürnberg, G.; Ender, K.: Post mortem changes in Ca<sup>2+</sup> transporting proteins of sarcoplasmic reticulum in dependence on malignant hyperthermia status in pigs. Mol. Cell. Biochem. 195 (1999a), 37-46

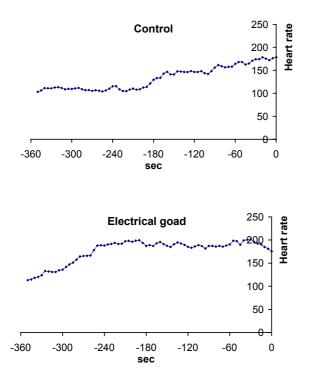
Küchenmeister, U.; Nürnberg, K.; Fiedler, I.; Kuhn, G.; Nürnberg, G.; Ender, K. Cell injury and meat quality of pig in the time period post mortem from two genotypes susceptible or resistant to malignant hyperthermia. Eur. Food Res. Technol. 209 (1999b), 97-103

Kerth, C.R.; Miller, M.F.; Ramsey, C.B.: Improvement of beef tenderness and quality traits with calcium chloride injections in beef loins 48 hours postmortem. J. Anim. Sci. 73 (1995), 750-756

O'Halloran, G.R.; Troy, D.J.; Buckley, D.J.; Reville, W.J.: The role of endogenous proteases in the tenderisation of fast glycolysing muscle. Meat Sci. 47 (1997), 187-210

Okamoto, A.; Suzuki, A.; Ikeuchi, Y.; Saito, M.: Effects of high pressure treatment on  $Ca^{2+}$  release and  $Ca^{2+}$  uptake of sarcoplasmic reticulum. Biosci. Biotechn. Biochem. 59 (1995), 266-270

Young, L.L.; Lyon, C.E.: Effect of calcium marination on biochemical and textural properties of peririgor chicken breast meat. Poultry Sci. 76 (1997),197-201



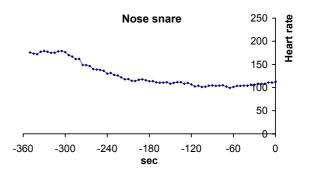


Fig. 1: Heart rate of pigs before slaughter. Control: the pigs could walk into the stunning pen without additionally stress; Nose snare: The pigs were immobilized for 5 min by a nose snare; Electrical goad: The pigs were stressed with an electrical goad for 5 min. The X-axis indicates the seconds before stunning (at 0 sec).



	Control	Nose snare	Goad	SE
Live weight at slaughter (kg)	108.4	107.7	105.8	1.10
Lean meat (%)	55.4	53.5	54.9	0.99
рН 45	6.28 a	6.24 a	5.86 b	0.11
Conductivity 24 h p.m. (mS)	5.70 a	6.15 a,b	8.49 b	0.88
Colour (Minolta) L*	49.9 a	49.2 a	53.7 b	1.22
Drip loss (%)	4.82 a,b	4.43 b	6.83 a	0.82
R-value	0.85 a	0.86 a	0.97 b	0.03

Tab. 1: Effect of different stress before slaughter on meat quality parameters \*

\* different letters indicate significant differences between experimental groups

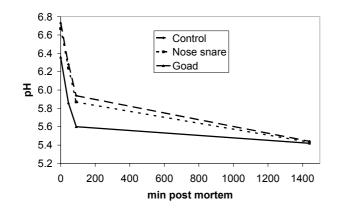


Fig. 2: Development of pH in the time course post mortem

Tab. 2:  $Ca^{2+}$  uptake rate (nM/min x homogenate protein) of *longissimus* homogenate, sampled at different intervals post mortem and immediately before stunning (shot biopsy). The calcium release channel (CRC) was closed by ryanodine treatment or basic without ryanodine treatment \*

	Closed CRC				Basic CRC			
	Control	Nose snare	Goad	SE	Control	Nose snare	Goad	SE
Biopsy	114.3 A	117.2 A	120.9 A	7.9	66.3 A	59.2 A	59.6 A	5.3
0 h	127.6 A	118.4 A	113.9 A	7.5	64.2 a A	56.6 a,b A	50.2 b B	4.5
45 min	105.0 a B	104.5 a A	84.7 b B	6.1	44.1 a B	41.7 a B	31.1 b C	3.4
4 h	92.9 a C	83.3 a,b B	71.4 b C	5.5	31.5 C	26.5 C	24.7 D	2.8

\* different lower case letters indicate differences between experimental groups different upper case letters indicate differences between sampling times

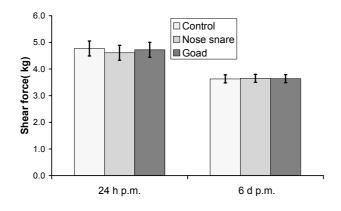


Fig. 3: Effect of stress on tenderness of *longissimus* samples, measured 24 h p.m. and following a 6 day storage