56th Annual Meeting of the European Association for Animal Production
June 5-8 2005, Uppsala, Sweden

The effect of driving pigs to stunning prior to slaughter on their stress status and meat quality

Cs. Ábrahám[1], M. Weber[1], K. Balogh[1], J. Seenger[1], M. Mézes[1], H. Febel[2] and E. Szűcs[1],*

[1]Szent István University, Gödöllő, Hungary,
[2]Research Institute for Animal Breeding and Nutrition, Herceghalom, Hungary

1. Abstract

Pigs of identical genotype at the HAL loci, i.e. NN were raised under same environmental conditions. Forty animals in all were transported from the pig farm to abattoir and slaughtered under commercial conditions. The aim was to establish effects of driving animals [with (Group A) or without electric goad (Group B)] to stunning. In blood samples taken 1 hour prior to stunning and during exsanguination, physiological parameters were determined as follows: cortisol, glucose, lactic acid. In addition, NEFA, MDA as well as indicators of the antioxidant defence system (ascorbic acid, GSH, GSHPx) were also analysed. In LD, following meat quality parameters (pH_{45}, pH_u, colour) were measured. Findings reveal significant differences between treatments only for cortisol (Group A: 122.75±48.11; Group B: 162.39±41.18; P<0.01), and NEFA (Group A: 0.52±0.22; Group B: 0.30±0.10; P<0.001). It was stated that the use of electric goad in driving pigs to stunning cannot be abandoned under commercial conditions. Electric goad helps in moving up animals to stunning faster, which results in lower stress level. For meat quality no significant differences were found. Thus, further evidence has been established that the detrimental effect upon meat quality in MHR-negative pigs is not as high as expected.

2. Introduction

The quality of pork covers several properties, which have to meet the increasing demands of consumers and processors. The main attributes of interest are color, pH and water-holding capacity, fat content and composition and also the oxidative stability.

The meat is very sensitive to oxidation leading to quality deterioration and loss of value. The tissues contain antioxidant defence systems. Superoxide dismutase, catalase and glutathione peroxidase are antioxidative enzymes contributing to the oxidative defence, as well as the fat-soluble α-tocopherol and ubiquinone, along with the water-soluble ascorbic acid and glutathione. The adequate activity of these defence systems are essential to develop appropriate meat quality.

The perimortal effects on meat quality and animal welfare are widely investigated. It is generally accepted, that the different environmental factors have a stronger impact than the genetic background. On the other hand the decreasing variance of Hal and RN genes, moreover the elimination of these genes makes it necessary to reevaluate the effect of environmental factors. Many studies showed differences in case of these effects when the experiments were carried out with halothane negative pigs.

3. Materials and methods

3.1. Experimental description

The present study was conducted at the Gyula Packing Plant Ltd (Hungary). A total of 40 pigs coming from one of the largest producer of the meat company were transported to the abattoir. All the animals were Dumeco hybrids. The pigs were assumed to be halothane negative (NN), even though they were not tested, as the gene has been practically eliminated from the breeds used by the pig farm.

The last feeding was 4 hours prior to transportation. The distance between the pig farm and the slaughter-house was 70 km, which lasted for 1.5 hours.

Right after the arrival and unloading the animals were divided into two groups driven into separate pens next to each other. The lairage time was 16 hours. One hour prior to stunning blood samples were taken. The groups were driven to stunning either with or without using electrical goad (EG).

The animals were stunned using electrical stunning method and slaughtered. During exsanguination the second series of blood samples were taken. The further processing method was carried out according to the local practice. After evisceration liver samples were taken from the *lobus caudatus*. The meat quality traits were measured two times: (1) 45 minutes after slaughtering, right after the slaughter value determination by Fat'o'Meater and (2) after chilling, at the 24th hour.

3.2. Biochemical analysis

The blood samples were taken from v. jugularis before and after transportation and during exsanguination. The following indicators of stress were determined from the samples: cortisol, NEFA, lactic acid and glucose.

The blood sampling tubes contained sodium-fluoride and potassium-oxalate. After centrifugation (10 min, 2500 rpm) the collected samples were stored at -20 °C until further analysis. For the cortisol assay a direct RIA method, developed in the laboratory of Szent István University, Faculty for Veterinary Sciences (Budapest, Hungary) was applied. For determination in blood plasma 1,2,6,7-3H-cortisol (TRK 407; Radiochemical Centre, Amersham, UK) and a highly specific polyclonal antibody raised against cortisol-21-HS-BSA in rabbit was used. The radiactivity was measured by Beckman Instrument Typ LS 1701 liquid scintillation counter.

NEFA, lactic acid and glucose was analysed with enzyme-colorimetric method using standard kits (*L-Lactate (PAP) kit*, Cat. No. LC 2389, Randox Laboratories Ltd., UK; *NEFA kit*, Cat. No. FA 115, Randox Laboratories Ltd. UK; Glucose kit (Cat. No. 40851, Diagnosticum Ltd.., Hungary).

Indicators of lipid peroxidation processes and antioxidant defence system measured were the followings: MDA (malondialdehyde), GSH (reduced glutathione), GSHPx (glutathione-peroxidase activity. The MDA, GSH and GSHPx values were determined from liver, blood plasma and red blood cells (RBC).

The blood samples were collected into heparine containing tubes, and centrifugated for 10 minutes (2500 rpm). The RBC hemolisate consisted of 1 vol RBC and 9 vol distilled water. Both hemolisate and blood plasma were stored at -20 °C until analyses.

The liver sample taking was followed by marking, packaging and storing at -20 °C. Immediately before analyses a sample of 0.5 g was homogenized in 9 vol of 4 °C physiological saline using Ultra Turrax (Donau Lab AG, Switzerland) homogenizer. For determination of MDA concentration the original homogenate, for the GSHPx, GSH and protein content the supernatant of the homogenate (centrifugation 10000 g, 20 min, 4 °C) were used.

MDA content describing the level of lipid peroxidation was carried out with 2-thiobarbituric acid determination. In case of blood plasma and RBC hemolizate the method of *Placer et al.* (1966) was followed, while the liver samples were analysed by the method described by *Mihara et al.* (1980).

The examination of antioxidant defence system included the determination of GSH concentration and GSHPx activity. The methods of *Sedlak et al.* (1968) and *Matkovics et al.* (1988) were used, respectively.

The protein content of blood plasma and RBC homogenizate was estimated by a modified biuret method (*Weichselbaum, 1948*), using egg protein (Randox Laboratories, UK) as standard. The protein content of liver homogenates was analyzed using Folin-Ciocalteau

phenol reagent (*Lowry et al.,* 1951), and bovine serum albumin (Reanal, Hungary) as standard.

3.3. Meat quality measurements

Meat quality parameters were measured two times: 45 minutes after slaughtering and after chilling at the 24th hour. The first measurement included pH and core temperature determination in the most valuable muscles: *m. longissimus dorsi* and *m. semimembranosus*.

24 hour after slaughtering we measured the pH and the color properties (L^*, a^*, b^*) of the loin. Temperature was measured with common meat industrial core thermometer, the pH measurement with a WTW 330 portable pH meter (WTW Gmbh., Germany) attached with WTW SenTix sp electrode and the color determination with a Minolta Chromameter CR-300 (Minolta Co., Japan) were carried out.

3.4. Statistical analysis

The data were analyzed with SPSS for Windows 10.0 program package, using t-test with independent samples and paired samples.

4. Results

The effect of method of driving animals to stunning is shown in *Table 1*. The duration of driving animals up to stunning was different. Systematic use of EG (column C) made driving faster (20 animals in 4 minutes). Without EG (column B) the driving was difficult and therefore lasted for longer time (20 animals in16 minutes). Driving pigs to stunning and stunning itself cause a heavy distress for the animals. As it is shown in column A, B and C, the cortisol level increased significantly in both groups at high level of probability from 101.72 nmol/l up to 162.39 ($P<0.001$) and 122.75 nmol/l ($P<0.01$), respectively). In without EG driven group (column B), the stress appeared to be serious as indicated by a motionless-state, flight, mounting and squealing. It seemed to be at a maximum at the entrance of the restrainer.

These observations are in agreement with the cortisol levels. In without EG driven group much lower cortisol concentrations were measured after bleeding, than in animals driven with the help of EG ($P<0.01$) which is evidence for lower stress level.

The lactic acid level increased from basal value (column A) approximately threefold higher independent of the way of driving (column B and C) in both groups ($P<0.001$), which is further evidence that driving animals to stunning requires heavy physical acitvity and muscle work. This statement can be supported by the fact there between lactic acid values no statistical differences were present ($P>0.05$). There was no measurable fatty acid mobilisation during the period of driving, irrespective of using EG, to stunning as shown by significantly lower and not higher NEFA content after stunning. Lipid peroxidation processes as measured by malondialdehyde also did not changed during that short period.

The method of driving animals up to stunning did not indicate any significant difference in case of meat quality (*Table 2*).

The effect of the treatment on the parameters measured in the liver is summarized in *Table 3*. The findings did not reveal any significant difference between the two groups.

Table 1 Effect of way of driving animals to stunning on blood parameters (means and standard deviation for blood parameters as well as results of statistical analysis)

Parameters	Before stunning (n=40)	After stunning without EG (n=20)	After stunning with EG (n=20)	Level of probability P		
	A	B	C	A-B	A-C	B-C
Cortisol (nmol/l)	101.72±32.41	162.39±41.18	122.75±48.11	***	**	**
Glucose (nmol/l)	4.57±0.30	4.67±0.41	4.74±0.242	NS	NS	NS
Lactic acid (nmol/l)	3.38±1.83	10.45±4.11	12.90±5.02	***	***	NS
NEFA (nmol/l)	0.68±0.24	0.30±0.10	0.52±0.22	***	**	***
MDA blood plasma (nmol/ml)	7.97±1.93	8.34±2.54	7.80±2.20	NS	NS	NS
MDA RBC hemolizate (nmol/g)	7,86±1,96	9,30±1,96	10,00±1,42	NS	NS	NS
GSH blood plasma (µmol/g protein)	3,41±0,71	2,95±0,71	3,26±0,87	NS	NS	NS
GSH RBC hemolizate (µmol/g protein)	2,94±0,35	2,91±0,33	3,12±0,25	NS	NS	*
GSHPx blood plasma (U/g protein)	4,53±0,67	4,61±1,17	5,23±1,58	NS	NS	NS
GSHPx RBC hemolizate (U/g protein)	4,37±0,55	4,33±0,42	4,93±0,60	NS	NS	***

NS P>0.05
* P<0.05
** P<0.01
*** P<0.001

Table 2 Effect of way of driving animals to stunning on blood parameters (means and standard deviation for blood parameters as well as results of statistical analysis)

Parameters	After stunning without EG (n=20)	After stunning with EG (n=20)	Level of probability P
	A	B	A-B
pH_{45loin}	6.02±0.26	5.96±0.22	NS
pH_{45ham}	6.15±0.37	5.98±0.34	NS
T_{45ham}	40.71±0.56	40.52±0.37	NS
pH_{uloin}	5.54±0.12	5.51±0.13	NS
L^*_{loin}	41.24±3.91	41.62±4.07	NS
a^*_{loin}	7.40±1.03	7.51±0.83	NS
b^*_{loin}	1.33±1.03	1.56±1.04	NS

NS P>0.05

Table 3 Effect of way of driving animals to stunning on parameters measured in the liver (means and standard deviation for blood parameters as well as results of statistical analysis)

Parameters	After stunning without EG (n=20)	After stunning With EG (n=20)	P
	A	B	A-B
MDA	7,31±2,99	7,63±3,41	$0,752^{NS}$
GSH	1,23±0,51	1,19±0,40	$0,753^{NS}$
GSHPx	1,90±0,30	1,80±0,29	$0,308^{NS}$

5. Conclusions

It is difficult to decide to use or abandon the electric goad. Many references notice that the use of this equipment increases the muscle metabolism and accelerate the decline in pH. From the point of view of animal welfare the electric goad is also treated as something detrimental. On the other hand we have to take into consideration that in case of large-scale abattoirs it is almost impossible to drive up animals to stunning without electric goad as fast as it is required by the slaughtering and processing technology. For the future, only automated systems have to be considered for application, where the animals are driven up to stunning without human intervention.

Furthermore, if driving animals to stunning is prolonged, it can increase the stress level, because the hypothalamus-pituitary-adrenal cortex axis (HMA- axis) has enough time to being activated. Increased concentration of cortisol at the moment of stunning is expressly disadvantageous for the further processing, because this stress hormone has vasoconstrictive effect, which can result in inadequate exsanguinations.

Further conclusion could be that during large-scale, commercial slaughtering and processing the pre-slaughter factors have much lower effect on meat quality than among experimental circumstances. Sometimes the processing technology (singe, dehairing, etc.) increase the carcass temperature to extremely high (the core temperature can be above 41-42 °C), which accelerate the pH decline significantly. In our opinion these major factors eliminate the effect of pre-slaughter handling.

The perimortal effects on meat quality and animal welfare have been investigated in comprehensive studies. It is generally accepted, that the different environmental factors have a stronger impact than the genetic background. On the other hand the decreasing variance of Hal and RN genes, moreover the elimination of these genes makes it necessary to reevaluate the effect of environmental factors. Many studies showed differences in case of these effects only when the experiments were carried out with halothane negative pigs. For the future, one of the most important tasks to understand how post slaughter processing and pres-laughter factors interact in relation to pork quality. We have to give a huge number of tools to control pork quality and hereby meat quality demands for tomorrow.

6. References

Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall, R.J. (1951): Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry, 193. 265-275.

Matkovics, B., Szabó, L., Sz. Varga, I. (1988): Lipidperoxidáció és redukált glutation anyagcsere enzimek aktivitás meghatározása biológiai mintákban. Lab.Diagn. 15. 248-250.

Mihara, M., Uchiyama, M., Fukuzawa, K. (1980): Thiobarbituric acid value of fresh homogenate of rat as parameter of lipid peroxidation in ageing, CCl_4 intoxicati- on and vitamin E deficiency. Biochem. Med. 23. 302-311.

Placer, Z.A., Cushman, L.L., Johnson, B.C. (1966): Estimation of product of lipid peroxidation (malon dialdehyde) in biochemical systems. Anal. Biochem. 16. 359-364.

Sedlak, I., Lindsay, R.H. (1968): Estimation of total, protein-bound and non-protein sulfhydryl groups in tissues with Ellmann's reagent. Anal.Biochem. 25. 192-205.

Weichselbaum, T.E. (1948): An accurate and rapid method for the determination of protein in small amounts of serum and plasma. Am. J. Clin. Pathol. 16. 40-43.