INVESTIGATION OF PHYSIOLOGICAL PARAMETERS INDICATING STRESS STATUS IN SLAUGHTER PIGS DURING TRANSPORT AND LAIRAGE

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Background

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.
The optimum lairage time for pigs is a question of compromise. Very short lairage time does not allow sufficient time for recovery leading to a higher incidence of PSE-meat. No prolonged lairage time is recommended after a long-term transportation, because the animals are already exhausted, so the further lairage may increase the incidence of DFD-meat.

Objectives

- study the effect of transportation as a stress factor,
- the effect of transportation and lairage on the antioxidant defence system,
- effect of lairage time on physiological and meat quality traits.

Materials and methods

The present study was conducted at the Gyula Packing Plant Ltd (Hungary). A total of 40 Dumeco hybrid pigs were transported from one of the largest producer of the meat company were transported to the abattoir. 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.
Animal were fed 4 hours prior to transportation. They were driven out from the piggery to the waiting pen right before the blood samples were taken. For further identification, the animals were marked individually by ear tag. After sampling the animals were driven up to a three-decker lorry. The experimental animals traveled on the lowest level. The distance between the pig farm and the slaughter-house is 70 km, which lasted 1,5 hr for the lorry. After arrival the animals were weighted, which was followed by the second blood sample taking. Then the pigs were divided into two groups: one hour lairage time (Group A), and 16 hours lairage time (Group B) according to the local practice. Showering was used only in the case of the longer lairage.
After lairage the animals were stunned using electrical stunning method and slaughtered. During exsanguination the third 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.

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. Blood sampling tubes contained sodium-fluoride and potassium-oxalate. After centrifugation (10 min, 2500 rpm) the samples taken 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 were measured as follows: MDA (malondialdehyde), GSH (reduced glutathione), GSHPx (glutathione-peroxidase activity) and ascorbic acid. The MDA, GSH and GSHPx values were determined from liver, blood plasma and red blood cells (RBC). The concentration of ascorbic acid was analyzed in liver samples and blood plasma. 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.

For the ascorbic acid determination 1 ml blood plasma was added to 1 ml 10 % TCA (trichloric-acid) and followed by centrifugation (20 min, 3500 rpm). 0,5 ml of the supernatant was stored at -20 °C until the further analyses. A liver sample of 0,5 g was homogenized in 9 vol 5% TCA, which was followed by centrifugation (20 min, 3500 rpm). The analyses were carried out using the method described by *Omaye et al.* (1979).

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*. We registered the chilling loss, which was computed from the difference of carcass weight before (approx. 50 min after slaughtering) and after chilling. 24 hour after slaughtering we measured the pH and the color properties (L^*, a^*, b^*, Japanese color scale) 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.

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

Results and discussion

Transportation as a stress factor is considerable in case of properties measured. The significance values are summarized in *Table 1*.

The activation of hypothalamus-pitiutary-adrenal cortex (HPA) axis results in a higher cortisol level, which is followed by the increase of NEFA, glucose and lactic acid values. The state of antioxidant defence system is affected by transportation, which is demonstrated by reduced GSH level. However, the oxidative load is not so significant to enhance the activity of GSHPx, moreover both in blood plasma and RBC hemolizate decreasing has obsessed. The MDA concentration differs significantly only in case of RBC hemolizate. The transportation affects also the ascorbic acid concentration, although the level of significance is low ($P<0,05$).
The effects of lairage on biochemical parameters measured are summarized in *Table 2*. The data measured at arrival and during exsanguinations and the data of the two different lairage time were compared.
Both 1 hour and 16 hour lairage increases the level of lactic acid, glucose and ascorbic acid. No significant difference is revealed in case of cortisol and NEFA or in the most of the parameters characterizing the antioxidant defence system.
Statistical analysis did not reveal any difference between the two groups. Only the GSHPx activity measured in RBC hemolizate increases at a low significance level ($P<0,05$).
The results of the liver samples analysis are shown in *Table 2*. Significant differences were found in case of GSH and ascorbic acid. The level of lipid peroxidation increases, which is illustrated with the reducing MDA values, although it is not significant in consequence of high individual variance. No difference is experienced in case of GSHPx.
The results of meat quality measurements are summarized in *Table 3*. Significant differences were found in case of pH_{45} and core temperature, along with the chilling loss. No difference was revealed in any of the meat quality traits measured after chilling, at the 24th hour.

Considering the biochemical parameters measured, we can state that the transportation causes high stress for the animals. The activation of HPA-axis results in an increased cortisol level. This hormone has a key-role in the gluconeogenesis. It is conducive to transform some amino acids and other gluconeogenetic substances (lactic acid, proprionic acid, glycerin) to glucose and glycogen. Addition to the stimulation of gluconeogenesis, the cortisol inhibits glycolisis. The enhanced production and decreased metabolism of glucose resulted in approx. 25 % higher blood glucose level, in spite of the short transportation time. The catabolic effect of cortisol on lipid metabolism is also manifested. This physiological process is important to ensure the energy requirement of animals during stress. The effect of lipid mobilization is revealed in level of NEFA, which increased approx. 100 %.
The increase of lactic acid is a bit surprising. As it was mentioned above, one effect of cortisol is to enhance gluconeogenesis, which changes the lactic acid level negatively. This phenomenon can be explained by the high physical exertion caused by transportation and the surrounding animal handling. These physical loads can enhance the depletion of lactic acid from muscles to blood.
The effect of transportation on the antioxidant defence system is revealed. The considerable oxidative load results in lower level of GSH both in blood plasma and RBC hemolizate. These changes are not so significant to enhance the activity of GSHPx, moreover a slight decrease can be seen. The reduced level of ascorbic acid was induced by stress. The change can be explained by its role in antioxidant defence system, and in the synthesis of cortisol in adrenal cortex.
During 1 hour lairage the stress was further enhanced. It is shown by increasing cortisol level, although it was not significant. The lactic acid enhanced three-times higher during lairage, which means that driving to stunning creates a very stressfull situation. The 16 hour lairage has quite similar results. No significant change in cortisol comparing the arrival state with the exsanguinations state is revealed. The level of lactic acid and glucose enhances with a high degree, along with the other group. The NEFA level do not changes either of the two groups, remains at the level measured at time of arrival. Changes of antioxidant defence system are obsessed only in case of blood plasma, the GSH reduces during lairage.
No significant differences were revealed between the two different groups. Two conclusions might be drawn from the results. (1) It is possible, that the lairage conditions are not adequate, and it not ensures enough rest for the animals. (2) It is also possible, that driving to stunning, and the stunning itself causes such a high stress for the animals, which is enough to eliminate the positive effect of lairage. This is suggested by the biochemical parameters.
The results of the meat quality measurements are in agreement with the biochemical measurements. Even though the pH_{45} and temperature values were lower in case of Group B (the lactic acid values were also higher at the time of stunning), these differences were equalized up to the second measurements. Thus, no significant differences are revealed in case of pH_{24} and meat color.
The analyses carried out on liver samples resulted in differences for two parameters. Ascorbic acid reduces drastically, which is caused by the enhanced metabolism, and depletion to blood. The GSH also decreases

significantly. It is presumable that the large-scale changes in both parameters are caused by fasting. At the case of longer lairage the fasting time exceeded 20 hours, so the reduced GSH level is explainable with the lack of supply of amino acids required for GSH synthesis.
Summing up the results, we can conclude that transportation, lairage and driving to stunning cause a heavy distress effect for the animals. On the other hand, these factors do not cause such a high oxidative damage, which appears in the meat quality. Negative changes in the antioxidant defence system are shown, which is traceable in the level of glutathione and ascorbic acid. The lipid peroxidation processes are not influenced significantly by short-term transportation or lairage. The oxidative stability of liver after long lairage was highly reduced, thus, if it is planned to have further processing from the liver, long lairage and fasting is not recommended.

Conclusions

It can be concluded that when using halothane free animals, meat quality is largely independent of lairage time. It is important to underline, that the decision of lairage time is a question of compromise. In addition to meat quality it is influenced by hygiene and the question of work organization. It can be required to have the animal transportation on the day before slaughtering to have a safely continuous working of the abattoir.

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Table 1.: Effect of transport and lairage on biochemical parameters measured

Parameter	Transportation	Arrival- 1 hour lairage	Arrival- 16 hour lairage	1 hour lairage- 16 hour lairage
	P	P	P	P
Lactic acid	0.013^{*}	0.000^{***}	0.000^{***}	0.571^{NS}
NEFA	0.000^{***}	0.071^{NS}	0.378^{NS}	0.918^{NS}
Glucose	0.001^{***}	0.000^{***}	0.000^{***}	0.463^{NS}
Cortisol	0.001^{***}	0.572^{NS}	0.324^{NS}	0.946^{NS}
Ascorbic acid	0.019^{*}	0.007^{**}	0.001^{***}	0.360^{NS}
MDA (blood plasma)	0.482^{NS}	0.000^{***}	0.000^{***}	0.981^{NS}
GSH(blood plasma)	0.026^{*}	0.458^{NS}	0.004^{**}	0.083^{NS}
GSHPx (blood plasma)	0.021^{*}	0.911^{NS}	0.127^{NS}	0.250^{NS}
MDA (RBC)	0.000^{***}	0.358^{NS}	0.360^{NS}	0.032^{*}
GSH (RBC)	0.000^{***}	0.035^{*}	0.859^{NS}	0.279^{NS}
GSHPx (RBC)	0.000^{***}	0.975^{NS}	0.553^{NS}	0.040^{*}

Table 2.: Effect of lairage on parameters measured in liver

Parameter	1 hour lairage			16 hour lairage			P
	Mean	SD	cv %	Mean	SD	cv %	
MDA (µmolg/g)	2.229	1.359	60.96 %	3.558	2.299	64.61 %	0.108^{NS}
GSH (µmol/ g protein)	21.173	3.048	14.39 %	17.338	5.994	34.57 %	0.000^{***}
GSHPx (U/ g protein)	28.240	6.822	24.15 %	28.108	9.614	34.20 %	0.963^{NS}
Ascorbic acid (ug/g)	508.436	95.280	18.73 %	369.444	74.267	20.10 %	0.000^{***}

Table 3.: Effect of lairage on meat quality

Parameter	1 hour lairage		16 hour lairage		P
	Mean	SD	Mean	SD	
pH_{45ham}	6.495	0.160	6.201	0.272	0.000^{***}
pH_{45loin}	6.213	0.221	5.902	0.225	0.000^{***}
T_{loinj}	41.042	0.562	40.650	0.519	0.034^{*}
L^{*}	40.967	13.194	45.622	11.938	0.269^{NS}
a^{*}	5.018	2.566	3.831	1.546	0.099^{NS}
b^{*}	2.777	0.802	1.660	1.359	0.006^{*}
pH_{24}	5.606	0.077	5.651	0.098	0.132^{NS}