

# Slaughter value, meat quality, creatine kinase activity and cortisol levels in the blood serum of growing-finishing pigs slaughtered immediately after transport and after a rest period

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## Abstract

The experimental materials comprised 44 hybrid [♀ (Polish Large White x Polish Landrace) x ♂ Duroc] growing-finishing pigs. The animals were randomly divided into two groups: 24 pigs were slaughtered immediately after transport and 20 pigs were slaughtered after a 24-hour rest period in the lairage. The meat content of pork carcasses, carcass dressing percentage, the proximate chemical composition, physicochemical and sensory properties of meat and shear force values were determined. Serum creatine kinase activity and cortisol levels were determined in blood samples collected before transport and during carcass bleeding.

Pigs slaughtered immediately after transport, compared with those slaughtered after a 24-hour rest period, were characterized by a higher meat content of the carcass and a higher carcass dressing percentage. Pre-slaughter handling had no effect on pork quality. The incidence of normal-quality meat, partially PSE (pale, soft, exudative) meat and PSE meat was similar in both groups. Chemical analysis showed that the content of dry matter, total protein, fat and minerals in meat was comparable in both groups. As regards the functional properties of the pork, samples from the carcasses of pigs that had rested before slaughter had a higher contribution of the red color component. Meat from pigs slaughtered immediately after transport had more desirable sensory properties.

Pre-slaughter resting had a significant effect on those analyzed physiological parameters which were found to be good indicators of pre-slaughter stress. Serum creatine kinase activity and cortisol levels were higher in blood samples collected after transport (during carcass bleeding) than in samples collected before transport, pointing to a strong stress response of animals to pre-slaughter treatment. The decrease in serum cortisol levels in blood samples collected during bleeding from the carcasses of pigs slaughtered after a 24-hour rest period, compared with samples collected from animals slaughtered immediately after transport, suggests that rest before slaughter alleviated stress induced by pre-slaughter handling operations.

**Key words:** growing-finishing pigs, slaughter value, meat quality, creatine kinase, cortisol.

## Introduction

In the light of current knowledge, variations in the quality of pork meat result from a combination of factors, including production factors (breed, species, age, nutritional regime, housing and sanitary conditions, veterinary prevention), biological factors (individual susceptibility to production of collagen fibers and intramuscular fat) and pre-slaughter handling operations, i.e. loading, transport, unloading, fasting, pre-slaughter detention and slaughter method (Monin 1998, Candek-Potokar et al. 1999). The above procedures are non-specific stress factors that stimulate various organs, and increase the metabolic rate and body temperature. Functional disorders in animals subjected to pre-slaughter handling operations may decrease the quantity and deteriorate the quality of meat (Gispert et al. 2000). Sudden and powerful stress factors can lead to the rapid depletion of glycogen stores, excessive accumulation of lactic acid and a decrease in pH values, resulting in PSE meat (Fischer 2001). According to Grandin (1997), the responsibility for the qualitative deterioration of pork meat is shared 50% by the producer, and 50% by the personnel responsible for transport, livestock driving, animal slaughter and post-slaughter handling of meat. Tarrant (1992) argues that the last 24 hours before slaughter are crucial for the entire pork production cycle.

Inadequate pre-slaughter handling operations often elevate the blood levels of catecholamines (adrenaline and noradrenaline), adrenal cortex hormones (cortisol) and increase creatine kinase (CK) activity (Bradshaw et al. 1996). The blood levels of stress hormones, including cortisol, play an important role in evaluating the animals' susceptibility to environmental stress (Griffith and Minton 1992, Grandin 1997). Every stress stimulus, in particular psychological stress, leads to the excessive secretion of corticotropic hormones which have a suppressing effect on the immune system and lower the body's resistance to endogenous bacterial infections (De Jong et al. 2001). Creatine kinase activity is a vital indicator of stress sensitivity (Fitko et al. 1982, Barnett et al. 1984). Creatine kinase is an enzyme found in muscle tissue. The damage to muscle cells resulting from the strain inflicted on animals during pre-slaughter handling releases the enzyme into the bloodstream. Elevated levels of CK activity are indicative of the extent of tissue damage (Harris 1993). The intensity of the body's response to stress can be measured with the use of physiological factors to assess the effect of pre-slaughter handling on the quality and slaughter value of meat and to classify the stress-inducing character of animal rearing and veterinary practices.

The aim of this study was to compare the quality and the slaughter value of meat, creatine kinase activity and cortisol levels in the blood serum of grow-

ing-finishing pigs slaughtered directly after transport and after a 24-hour rest period.

## Materials and Methods

The experimental materials comprised 44 hybrid [♀ (Polish Large White x Polish Landrace) x ♂ Duroc] growing-finishing pigs that were fattened from around 30 kg BW to around 110 kg BW at the same fattening house. The animals were kept in individual metabolic cages and were fed a complete diet throughout the experimental period, in accordance with Pig Nutrient Requirements (Standards for feeding pigs 1993). Feed was served wet, and the pigs had free access to water. The complete diet contained 15.5% total protein, 0.82% total lysine, and 12.8 MJ metabolizable energy per kg of feed. Feed components were as follows: ground wheat, ground barley, soybean meal, rapeseed meal, and feed additives: minerals, vitamins and synthetic amino acids.

Before transport to the meat processing plant, blood was sampled from the jugular vein to determine creatine kinase (CK) activity and cortisol levels (the time of blood sample collection did not exceed 22 seconds) (Kowalski et al. 1996). Blood samples were collected again during carcass bleeding. Blood sample test tubes were transported to the laboratory in containers, on ice. Serum creatine kinase activity was determined by the kinetic spectrophotometric method proposed by the International Federation of Clinical Chemistry. Serum cortisol levels were measured by ECLIA electrochemiluminescence.

Growing-finishing pigs were transported over a distance of approximately 50 km, in late morning hours, at a temperature of around 25°C. The loading, transport and unloading of animals were carried out in accordance with the relevant regulations. The animals were randomly divided into two groups (sex ratio of 1:1): 24 pigs were slaughtered immediately after transport and 20 pigs were slaughtered after a 24-hour rest period in the lairage where they had free access to water.

Slaughter and post-slaughter processing were carried out in accordance with the relevant meat industry regulations. Carcass weight was determined and carcass lean meat content was estimated with the CGM device 45 minutes after stunning. Muscle tissue acidity (pH<sub>45</sub>) was measured in *musculus longissimus lumborum*, at the level of the 2<sup>nd</sup> and 4<sup>th</sup> lumbar vertebra.

The carcasses were stored at 2-4°C for approximately 24 hours. After chilling, samples of *m. longissimus dorsi* for laboratory analyses were collected from the right half-carcasses, from the region between the last thoracic vertebra and the 2<sup>nd</sup> lumbar vertebra. Approximately 48 hours post mortem, the following determinations were made: the chemical composi-

tion of meat (content of dry matter, fat, total protein, mineral compounds in ash form) by conventional methods (Rak and Morzyk 2002),  $\text{pH}_U$  (in meat homogenates, with a combination Double Pore electrode (Hamilton) and a 340i pH-meter equipped with a TFK 150/E temperature sensor (WTW), the water-holding capacity of meat (forced drip loss) by the Grau and Hamm method (Oeckel Van 1999), cooking loss (Honikel 1998), drip loss (Honikel 1998), meat color based on the values of CIELAB coordinates  $L^*$  (lightness),  $a^*$  (redness),  $b^*$  (yellowness), (CIE 1978), by the reflectance method, with a MiniScan XE Plus instrument (HunterLab), shear force (after heat treatment) using a Warner-Bratzler head (500 N, speed 100 mm/min.) attached to an Instron universal testing machine (model 5542), and the sensory properties of cooked meat on a five-point scale (PN-ISO 4121: 1998).

Statistical calculations were performed using the STATISTICA data analysis software system, ver. 8.0. The significance of differences between mean values in groups was estimated by a one-way analysis of variance and Duncan's test.

## Results

The studied hybrid growing-finishing pigs [♀ (Polish Large White x Polish Landrace) x ♂ Duroc] were slaughtered after different pre-slaughter detention periods. The first group comprised animals slaughtered directly after transport (24 head), and the second group included pigs slaughtered after a 24-hour rest period in the lairage (20 head). Live weight was 1.4 kg higher and hot carcass weight was 2.5 kg higher in the group of growing-finishing pigs slaughtered directly after transport (Table 1). The differences between the means of the above groups were statistically non-significant. An analysis of variance

pointed to highly significant differences in the percentage meat content of the carcass. The carcasses of pigs slaughtered directly after transport were marked by higher meatiness. A significantly higher carcass dressing percentage was also noted in the above group.

The duration of the pre-slaughter detention period did not have a significant effect on the chemical composition of the analyzed meat samples (Table 1). An insignificantly higher percentage content of dry matter, total protein, fat and ash was determined in meat from growing-finishing pigs rested for 24 hours before slaughter. The differences between the mean values of the above groups were statistically non-significant.

The average pH values of meat determined 45 minutes ( $\text{pH}_{45}$ ) and 48 hours ( $\text{pH}_U$ ) after slaughter were similar in the analyzed groups (Table 2). Based on the threshold  $\text{pH}_{45}$  values given by Kortz (2001), meat samples were divided into the following quality groups:  $\text{pH}_{45} > 6.3$  – normal-quality pork,  $\text{pH}_{45} = 6.0 - 6.3$  – partially PSE pork,  $\text{pH}_{45} < 6.0$  – PSE pork. In the majority of pork samples from both studied groups,  $\text{pH}_{45}$  values exceeded 6.3. Meat with normal pH values had a 66.66% share of the group of growing-finishing pigs slaughtered directly after transport and a 70.00% share of the group of animals slaughtered after a 24-hour rest period. 29.16% of meat samples from pigs slaughtered immediately after transport were characterized by  $\text{pH}_{45}$  values in the range of 6.0 – 6.3 which are indicative of PSE meat. In the group of growing-fattening pigs slaughtered after a rest period, only 25.00% of meat samples fell in the above category. An analysis of acidification of *m. longissimus lumborum* revealed one sample of PSE meat in each group.

An analysis of color parameters (CIELAB) of the studied meat (Table 2) did not show significant variations between the average values of  $L^*$  (lightness) and  $b^*$  (yellowness). The color of meat from grow-

Table 1. Live weight at slaughter, hot carcass weight, meat content of carcass, carcass dressing percentage and the chemical composition of meat from growing-finishing pigs.

Specification	Pre-slaughter detention time			
	immediate slaughter		slaughter after 24 h	
	$\bar{x}$	s	$\bar{x}$	s
Live weight at slaughter (kg)	110.48	5.32	109.10	7.42
Hot carcass weight (kg)	85.29	4.57	82.74	6.12
Meat content of carcass (%)	58.32 <sup>xx</sup>	1.61	56.58	2.51
Carcass dressing percentage (%)	77.19 <sup>*</sup>	1.56	75.83	1.79
Dry matter (%)	24.83	1.08	25.42	1.06
Total protein (%)	22.53	0.8	22.98	0.76
Fat (%)	1.80	0.78	1.94	0.89
Ash (%)	1.14	0.05	1.15	0.03

<sup>\*</sup> –  $P \leq 0.05$ ; <sup>xx</sup> –  $P \leq 0.01$

Table 2. Physicochemical properties of meat from growing-finishing pigs.

Specification	Pre-slaughter detention time			
	immediate slaughter		slaughter after 24 h	
	$\bar{x}$	s	$\bar{x}$	s
pH <sub>45</sub>	6.29	0.16	6.40	0.19
pH <sub>U</sub>	5.45	0.13	5.43	0.10
L* (lightness)	57.76	2.54	56.36	2.98
a* (redness)	6.24	1.13	6.96 <sup>x</sup>	0.96
b* (yellowness)	14.35	1.02	14.36	1.07
Water-holding capacity (cm <sup>2</sup> )	7.42	1.45	7.10	0.81
Cooking loss (%)	36.06	2.58	36.00	2.14
Drip loss (%)	6.95	1.56	6.74	2.12

<sup>x</sup> – P ≤ 0.05; <sup>xx</sup> – P ≤ 0.01

Table 3. Sensory properties and shear force value of meat from growing-finishing pigs.

Specification	Pre-slaughter detention time			
	immediate slaughter		slaughter after 24 h	
	$\bar{x}$	s	$\bar{x}$	s
Juiciness (points)	4.81 <sup>xx</sup>	0.32	3.93	0.59
Tenderness (points)	4.67 <sup>xx</sup>	0.43	3.87	0.77
Palatability – intensity (points)	4.89 <sup>xx</sup>	0.21	4.60	0.35
Palatability – desirability (points)	4.89 <sup>xx</sup>	0.21	4.52	0.38
Shear force (N)	27.52	6.86	29.75	5.11

<sup>x</sup> – P ≤ 0.05; <sup>xx</sup> – P ≤ 0.01

Table 4. Creatine kinase activity and cortisol levels in the blood serum of growing-finishing pigs.

Specification	Stat. meas.	Blood sampling				Statistically significant differences
		before transport		during carcass bleeding		
		(A) immediate slaughter	(B) slaughter after 24 h	(C) immediate slaughter	(D) slaughter after 24 h	
Creatine kinase (U/l)	$\bar{x}$	1593.0	1675.5	8709.75	8914.3	A, B < C, D <sup>xx</sup>
	s	693.64	1449.57	5833.59	8655.38	
Cortisol (µg/dl)	$\bar{x}$	3.39	4.07	26.54	15.44	A, B < C, D <sup>xx</sup> C > D <sup>xx</sup>
	s	1.83	2.89	15.02	7.46	

<sup>xx</sup> – P ≤ 0.01

ing-finishing pigs slaughtered after a rest period was marked by a higher contribution of redness (statistically significant at P ≤ 0.05). The average volume of forced drip, cooking loss and natural drip from the studied meat samples was similar in both groups.

The juiciness, tenderness and palatability of meat from pigs slaughtered directly after transport received very high scores (on a five-point scale) (Table 3). The difference between the average results noted in the

above group and the group of growing-finishing pigs slaughtered after a 24-hour rest period was highly significant. The tenderness of meat samples from pigs slaughtered directly after transport was highly satisfactory, and this was demonstrated by lower shear force values of cooked meat (27.52 N) in comparison with the other group of animals. However, no statistically significant differences were noted between the mean values in the above groups.

Creatine kinase activity and cortisol levels were determined in the blood serum of the analyzed pigs (Table 4). CK activity was highly significantly higher in the blood samples collected during carcass bleeding than in the samples collected before transport. Elevated CK activity levels were noted in the blood serum of growing-finishing pigs slaughtered immediately after transport and pigs slaughtered after a 24-hour rest period. Identical correlations were observed in an analysis of blood serum cortisol levels. An additional analysis of variance demonstrated highly significant differences in cortisol levels in samples collected during carcass bleeding. Cortisol levels decreased from 26.54 µg/dl in the blood of growing-finishing pigs slaughtered directly after transport to 15.44 µg/dl in the blood of animals slaughtered after a rest period.

## Discussion

Breed improvement efforts and long-term selection for a fast growth rate during the fattening period and high carcass meatiness have produced animals with a low adaptive potential and high sensitivity to environmental changes. All pre-slaughter operations inflict high levels of stress on the handled animals. Stress factors lead to quantitative loss and adverse changes in the quality and the storage life of meat (Prost 1981). A clear deterioration in the slaughter value and the quality of meat from growing-finishing pigs is observed when health and nutritional requirements are disregarded prior to transport, when transport is prolonged and when the animals are kept in the lairage for excessive periods of time.

The duration of pre-slaughter rest is difficult to determine, and is subject to various factors, including species, breed, age and individual traits. For the physiological balance to be restored, the animals have to be provided with adequate housing conditions in the lairage. According to popular opinion, the pre-slaughter rest period should correspond to the duration of transport. It should be noted, however, that a prolonged pre-slaughter rest period can lead to decreased carcass weight and lower meat quality.

Slaughter value is largely determined by the lean meat content of the carcass and the carcass dressing percentage. In the group of hybrid growing-finishing pigs [♀ (Polish Large White x Polish Landrace) x ♂ Duroc] slaughtered directly after transport, the lean meat content of the carcass was 1.7% higher and the carcass dressing percentage was 1.4% higher than in the group of animals slaughtered after a 24-hour rest period. These results seem to validate the view that animals are not only unable to rest in the lairage, but due to heightened stimulation, their metabolic rates increase, leading to a drop in meat quality and body weight loss (De Smet et al. 1996, Warris et al. 1998).

An analysis of the chemical composition of meat samples from the studied animal groups did not reveal significant differences. A tendency for a higher percentage share of dry matter, total protein and fat was noted in the growing-finishing pigs slaughtered after a rest period. The chemical composition of meat samples investigated in this experiment and the chemical composition of meat from growing-finishing pigs analyzed in other studies was similar (Koćwin-Podsiadła et al. 2004).

The processing suitability and the eating quality of meat are evaluated mainly based on its physicochemical properties, which affect the meat's appearance, shelf life and the sensory attributes of the final product (Kortz 2003). The pH value resulting from post-slaughter glycolysis is a key indicator of meat quality because it affects the eating quality (tenderness, juiciness), technological properties (color, water-holding capacity) and microbiological stability of meat (Guignot et al. 1994, Laack van et al. 2001). The pH values determined in the studied meat samples 45 minutes and 48 hours after slaughter were similar in the investigated groups and were within the norms for normal-quality meat, cited by other authors (Kortz 2001).

Various groups of meat quality are determined based on the rate of processes that lower or stabilize pH values in muscles after slaughter. A rapid drop in muscle pH after slaughter leads to the occurrence of PSE (pale, soft, exudative) meat – the defect most commonly encountered in pork. The quality of pork is significantly affected by interactions between environmental and genetic factors. The majority of defects result from intensive selection efforts aimed at producing the most desirable meatiness traits. These efforts have contributed to the frequent occurrence of genes conditioning qualitative defects in meat. An increase in the meat content of the carcass is accompanied by lower resistance to stress. In certain circumstances, the above may lead to qualitative changes and the development of PSE meat. There is an unquestionable correlation between meat quality and pre-slaughter rest. Pre-slaughter handling may intensify mechanisms that contribute to the occurrence of meat defects (Fischer 2001). In this study, the share of normal-quality meat, partially PSE meat and PSE meat was similar in both groups. Different pre-slaughter handling methods did not lead to variations in meat quality. The satisfactory results noted in this experiment could be attributed to the Duroc breed which was used as a paternal component in the cross-breeding process. Duroc pigs are characterized by high resistance to stress and ease of adaptation, and the above factors contribute to the high quality of their meat. It is one of the few breeds that has a clear heterosis effect on vital production parameters when crossed with other breeds.

Color is one of the key properties of meat that affect the consumers' decision to buy a given product (Brewer et al. 2002, Kennedy et al. 2004). Based on a visual evaluation of this trait, consumers form opinions on the freshness and eating quality of meat (Mancini and Hunt 2005). The average color parameter values ( $L^*a^*b^*$ ) noted in this study point to variations in the contribution of redness ( $a^*$ ) subject to the duration of the pre-slaughter rest period. Lower values of parameters  $L^*$  and  $b^*$  than those noted in this study were reported by Bee et al. (2006) and Cilla et al. (2006), while lower values of parameter  $a^*$  can be found in the work of McCann et al. (2008). Bee et al. (2006) observed similar values of  $a^*$  to those determined in this experiment, while higher values of this parameter were reported by Cilla et al. (2006).

Water-holding capacity is yet another factor affecting the processing suitability of meat (Oeckel van et al. 1999). Water-holding capacity determines the volume of meat weight loss during storage and the meat's ability to retain water during heat treatment (Aaslyng et al. 2003). In this study, the levels of forced drip, cooking loss and natural drip were measured to determine the water-holding capacity of the investigated meat samples. An analysis of the effect that various pre-slaughter handling methods had on the water-holding capacity of meat did not reveal significant differences between groups, and the results indicate that the studied meat samples were characterized by a highly satisfactory water-holding capacity.

Consumer perceptions rely mostly on the sensory attributes of meat. The results of many research studies indicate that meat from hybrid growing-finishing pigs crossed with the Duroc breed is characterized by juiciness, tenderness, and a desirable flavor and aroma. The above is most probably due to the high intramuscular fat content of meat from Duroc pigs (Fernandez et al. 1999, Suzuki et al. 2005, Cilla et al. 2006), which improves meat quality. An evaluation of the sensory properties of meat subject to the duration of the pre-slaughter period showed clear differences in the juiciness, tenderness and palatability of meat. Meat from growing-finishing pigs slaughtered directly after transport received higher scores for the above attributes. Meat tenderness results correspond to shear force values. No significant differences were reported between the average shear force values subject to the duration of the pre-slaughter rest period, but the group of pigs slaughtered after a 24-hour rest period showed a tendency for higher values of this attribute.

Creatine kinase present in the cytoplasm and mitochondria, mainly in the muscles, brain and heart tissue, catalyzes the breakdown of phosphocreatine in the presence of ADP to creatine and ATP. The above reaction is reversible, and it takes place with the in-

volvement of  $Ca^{2+}$  and  $Mg^{2+}$ . Phosphocreatine is the main source of energy for muscle contraction. The total activity of creatine kinase increases significantly in consequence of skeletal muscle damage, inflammations, ischemia and injuries caused by excessive strain. Owing to the short half-life of creatine kinase, heightened levels of CK activity are an indicator of recent muscle damage (Procajlo 2006) which can take place due to acute stress inflicted on the animals during pre-slaughter handling. Increased blood serum cortisol levels are also an indicator of stress response in animals (Agnes et al. 1990) resulting from the stimulation of the sympathetic and parasympathetic nervous system and the hypothalamic-pituitary-adrenal axis. The above stimulates the adrenergic system to produce catecholamines, and enhances the secretion of steroid hormones, mainly cortisol, from the adrenal cortex (Zavy 1992). In this study, a significant increase in creatine kinase activity (more than five-fold) and cortisol levels in blood samples collected after transport (during carcass bleeding) in comparison with blood samples obtained before transport suggests that pre-slaughter handling operations induced an intensive stress response in the investigated animals. CK activity in blood samples collected during carcass bleeding (8709.75-8914.3 U/l) was significantly higher than the referenced values (50-3531 U/l) (Winnicka, 1997). In comparison with blood samples collected from animals slaughtered immediately after transport, the decrease in serum cortisol levels in blood samples collected during bleeding from the carcasses of pigs slaughtered after a 24-hour rest period suggests that rest before slaughter restores the body's physiological balance and alleviates the stress induced by pre-slaughter handling (Gispert et al. 2000, Fischer 2001).

## Conclusions

Pigs slaughtered immediately after transport, compared with those slaughtered after a 24-hour rest period, were characterized by a higher (approximately 1.7%) meat content of the carcass and a higher (approximately 1.4%) carcass dressing percentage. Pre-slaughter handling had no effect on pork quality. The incidence of normal-quality meat, partially PSE meat and PSE meat was similar in both groups. Chemical analysis showed that the content of dry matter, total protein, fat and minerals in meat was comparable in both groups. As regards the functional properties of pork, samples from the carcasses of pigs that rested before slaughter had a higher contribution of the red color component. Meat from pigs slaughtered immediately after transport had more desirable sensory properties (juiciness, tenderness, palatability).

Pre-slaughter resting had a significant effect on the analyzed physiological parameters which were found to be good indicators of pre-slaughter stress. Serum creatine kinase activity and cortisol levels were higher in blood samples collected after transport (during carcass bleeding) than in samples collected before transport, pointing to a strong stress response of animals to pre-slaughter treatment. The decrease in serum cortisol levels in blood samples collected during bleeding from the carcasses of pigs slaughtered after a 24-hour rest period, compared with samples collected from animals slaughtered immediately after transport, suggests that rest before slaughter alleviated stress induced by pre-slaughter handling operations.

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