ELLEN HAMBRECHT

Critical pre- and postslaughter factors in relation to pork quality





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Critical pre- and postslaughter factors in relation to pork quality

Kritieke factoren omtrent het slachten in relatie tot de kwaliteit van varkensvlees

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Abstract

There exists a large variation in color and water-holding capacity of pork which gives rise to complaints by both producers and consumers. This thesis deals with factors related to pork processing plants that have the potential to influence these two quality attributes. Effects of handling during the preslaughter period, stunning method, and rate of chilling on color and water-holding properties of pork were studied. Particular attention was paid to interactions between the various pre- and postslaughter handling factors. Stress in the immediate preslaughter period was identified as the most critical among the factors studied regarding pork quality. Suboptimal transport and lairage conditions as well as high muscle glycogen levels at the moment of slaughter aggravated the negative effects of high preslaughter stress. It was concluded that first measures to improve pork quality should aim at avoiding or reducing stress in the immediate preslaughter period.

Contents

Chapter 1 | **1**

General introduction

Chapter 2 | **11**

Effect of processing plant on pork quality

Chapter 3 | **25**

Rapid chilling cannot prevent inferior pork quality caused by high preslaughter stress

Chapter 4 | **39**

Preslaughter stress and muscle energy largely determine pork quality at two commercial processing plants

Chapter 5 | **57**

Negative effects of stress immediately before slaughter on pork quality are aggravated by suboptimal transport and lairage conditions

Chapter 6 | **75**

Preslaughter handling effects on pork quality and glycolytic potential in two muscles differing in fiber type composition

Appendix to Chapter 6 | **93**

Effects of various preslaughter handling treatments on the color of different pork muscles

Chapter 7 | **103**

General discussion

Summary / Samenvatting / Zusammenfassung / Resumen | **125**

Acknowledgements | **143**

Curriculum vitae | **149**

General introduction

1

The sensory quality is still the most important determinant of the image of fresh meat (Schifferstein et al., 1998). At the moment of purchase, the color of pork is a crucial attribute in the quality assessment (Becker, 2000). Water-holding properties are of great importance, because retention and gains or losses of water affect the weight and thus the economical value of pork products. Additionally, the content and distribution of water within muscles may affect the visual appearance as well as other sensory characteristics such as tenderness and juiciness of meat (Gault, 1985; Offer and Knight, 1988). Therefore, all studies reported within this thesis focus on the color and water-holding capacity of pork. Color and water-holding capacity may be influenced by a variety of factors. This thesis deals with the effects of transport, lairage and stress level immediately before slaughter, the stunning method used, and the rate of carcass chilling. After a brief history of meat consumption, some developments of the last centuries related to the aforementioned factors are highlighted.

A brief history of pork processing and consumption

Pork consumption

Historically, meat consumption was closely related to the wealth of the population. In the late Middle Ages, labor scarcity, due to the diminished population after the plague, resulted in higher wages in the cities and increased purchasing power. The relative economical wealth resulted in a total meat consumption as high as 100 kg per head (Abel, 1981; for comparison: in 2003, total meat consumption in the EU amounted to 90 kg per head; Gira Meat Club, 2003). In the following centuries, the rise in population stimulated the use of land for crop production and reduced the function of livestock to tractive power and manure production. At the beginning of the 19th century, meat consumption had fallen to approximately 16 kg per head (Abel, 1981). Since around 1850, meat consumption has increased again. The enormous growth in grain production in the United States after the civil war, on the one hand, and the invention of artificial fertilizers, on the other, reduced feed prices and enabled the intensification of animal production (Abel, 1981). Especially pork production grew rapidly as a result of corn imports from the United States. Presently, in highly industrialized countries such as the USA, the Netherlands, and Germany, pork consumption per capita is more or less stable at approximately 30 kg, 42 kg, and 55 kg, respectively (Gira Meat Club, 2003), and depends much less on the purchasing power or pork prices. On the other hand, in rapidly growing economies, such as for example China, pork consumption experienced an almost 80%-increase, from 19 kg to almost 34 kg per capita, during the last ten years (Gira Meat Club, 2003). A similar relationship between wealth and meat consumption, but the opposite trend, is seen in countries such as Russia or the Ukraine, where economical difficulties are reflected in a fluctuation and an overall decrease in pork consumption during the last decade (Gira Meat Club, 2003). In wealthy economies, pork consumption is increasingly influenced by aspects such as healthiness,

diet variation, and ethical concerns (Lister, 1990) rather than by prices. However, within the lower income groups, also in these societies there appears to exist a relationship between purchasing power and meat consumption (Becker, 2000).

Pork processing

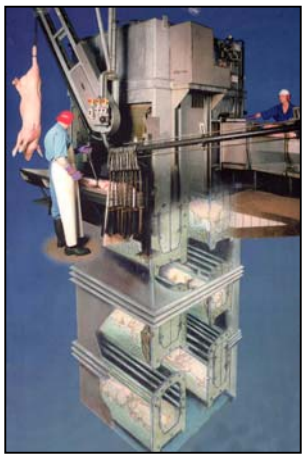
Preslaughter handling. The progressive urbanization in the 19th and 20th century called for a more regulated meat supply, mainly from a hygienic point of view (Koolmees, 1997). In the course of the 19th century, the methods of animal transport and slaughtering in the municipal slaughterhouses in the cities were increasingly criticized by animal conservationists. Excessive long periods without feed and water and rough handling at loading and unloading, resulting in bone fractures and other injuries, are some examples of cruelties to slaughter animals that were not uncommon at that time (Koolmees, 1997). The first regulations to improve the situation appeared at the end of the 19th century (e.g. Amsterdam, 1887): exhausted animals were not to be slaughtered directly after arrival but had to be given rest in order to recover (Koolmees, 1997). Today, the increasing concentration in pork production with fewer and larger processing plants as well as the free movement of animals within the EU from one member state to another increases the transport distances. EU directives aim at adequate preslaughter handling (e.g. EC Directive 1991; EC Directive 1993) but not all recommendations are implemented in national laws yet. In the Netherlands, traditionally ahead with welfare legislation, maximum transport duration is still 24 hours. However, there are recommendations regarding, for example, loading facilities, loading density, bedding, and access to food and water. Without feed, lairage duration at the processing plant is restricted to four hours; after that pigs must be fed and watered (Dutch Product Board, 2004; personal communication).

Figure 1. 'Traditional' pig transport (East Asia, left) and modern transport (right).

(1917)

Modern electrical stunning (Stork)

'Pig trap' (1921)



Modern gas stunning (Butina)

(1930)

Figure 2. Examples of early stunning of pigs, with and without restraint (Archive of the Department of Public Health and Food Safety, Faculty of Veterinary Medicine, Utrecht University, The Netherlands) and of modern electrical and gas stunning.

Stunning. In the 19th century, the slaughter of animals without preceding stunning was very common. Sticking without prior stunning was not always leading to a rapid death and pigs were reported on occasions to struggle, at apparent consciousness, for a period of three to four minutes (Galsworthy, 1912; cited by Gregory, 1985). Moreover, the mallet and the pole axe, the earliest stunning devices, were not really reliable in producing an effective stun. Howarth et al. (1925; cited by Gregory, 1985) reported that 100 pigs required 155 blows with a pole axe to produce unconsciousness. In the Netherlands, the first regulations concerning stunning methods were specified by laws at the beginning of the 20th century. From the same time period date the first experiments with innovative stunning methods, mainly electrical and CO_2 stunning (Blomquist, 1957; Gregory, 1985; Koolmees, 1997), as well as the development of

restrainers to facilitate secure and correct stunning (Koolmees, 1997). The captive bolt pistol, the successor of the mallet and the pole axe, is not used anymore in pigs in commercial slaughtering, because it produces unduly violent convulsions, which make carcass handling difficult and impair pork quality. Nowadays, in modern pork processing plants, electrical or CO_2 stunning systems are employed. The most common method is electrical stunning in various forms (manual or automated, low or high voltage etc.). In the Netherlands, Spain, and the USA, for example, there are only few plants employing CO_2 stunning. In Denmark, on the other hand, pigs are almost exclusively stunned by CO_2. While there still is much debate about the effects of the method of stunning regarding quality and welfare aspects, it is noteworthy that any stunning method is inferior to slaughter without stunning regarding pork quality (Overstreet et al., 1975).

Chilling. Carcass chilling after slaughter improves pork quality characteristics such as color and water-holding properties (Offer, 1991). The major benefits and reasons for the refrigeration of meat, however, are related to its preservative effect. The earliest refrigeration method in meat processing plants was ice, stored in overhead lofts with flues facilitating the circulation of the cold air. By 1914, in most American packing plants the ammonia compression system was installed with a refrigeration capacity of more than 90,000 tons per day (Krasner-Khait, 2002). With today's variety of chilling systems, from cold storages operating at around 4°C to blast chilling tunnels with temperatures as low as -30°C, a wide range in the rate of temperature decline in the carcass can be achieved.

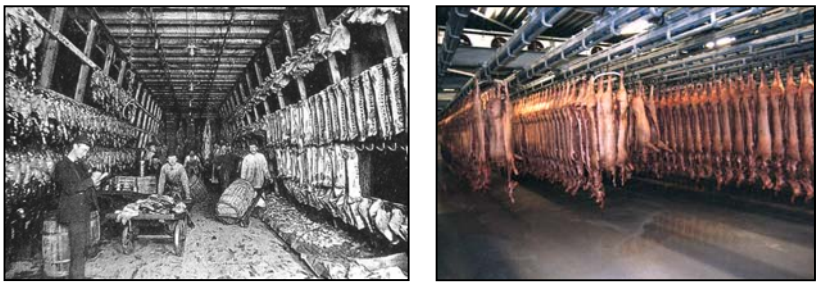


Figure 3. Meat storage in Chicago in 1892 (left) and modern cold storage (right).

It is obvious that the intensification of pork production has tremendously affected the way in which pork is produced. The changes were, however, not restricted to the production methods alone but extended to pig breeding. In the following, a short overview is given on the consequences of traditional breeding strategies.

Genetic selection

The main purpose of pig breeding has been to increase lean meat content, improve feed efficiency and growth rate, and reduce carcass fat content. As a result, genetic selection had profound effects on the physical constitution of the pig. The increase in muscle mass is the result of an increase in diameter rather than number of muscle fibers (Weiler et al., 1995), resulting in a reduced capillarization and thus oxygen supply. Moreover, there has been a gradual shift towards more glycolytic fiber types (Essén-Gustavsson and Lindholm, 1984; Weiler et al., 1995). The dependence on a predominantly anaerobic-glycolytic metabolism has transformed the former 'endurance-athlete' into an animal that has little staying power (Essén-Gustavsson and Lindholm, 1984). The lack of physical exercise in current husbandry systems seems also to play a role, as Bünger et al. (1977) showed that wild pigs fed and held under similar conditions as domestic pigs showed only a slightly better physical performance in a treadmill test compared with domestic pigs. An important issue in modern pig breeding represented the so-called halothane gene (a point mutation in the ryanodine receptor; Fuji et al., 1991) which is associated with specific breeds such as the Pietrain. This gene is not only correlated with a very high degree of muscling but also with a hypersensitivity to various stressors, resulting in more transport deaths and impaired pork quality (for review see Klont, 1994). The development of the halothane test by Eikelenboom and Minkema (1974) and later a DNA test (Fujii et al., 1991) allowed the exclusion of these pigs from breeding schemes. While homozygous halothane-positive end products are now very rare, carriers of the gene are still used due to their favorable muscling characteristics.

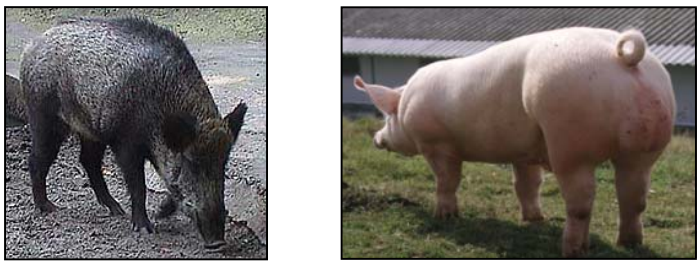


Figure 4. Wild boar (left) and domestic pig of a meat line (right).

Pork quality

The conditions around slaughter have very much improved during the last century, as has been pointed out before. However, after having been kept for its entire life in a confined space, the process of transport, lairage, and driving towards the stunning area is still both physically and psychologically very demanding for the pig. Moreover, the

high slaughter line speeds, which are enabled by certain stunning systems, may surpass the speed at which pigs voluntarily move. These physical and psychological stressors, associated with the slaughter process, may exceed the pig's coping capacity. Both ante- and postmortem muscle metabolism may be affected, which plays a major role in determining the color and water-holding properties of pork.

After slaughter, circulatory failure cuts both nutrient and oxygen supply to the muscle cells. In an attempt to maintain homeostasis, which is energy consuming, the remaining glycogen and glucose is then anaerobically degraded resulting in the accumulation of lactic acid and a concomitant pH decrease. This process continues until no more glycogen is available or until the decreasing muscle pH and low temperatures inhibit the activity of the enzymes involved.

Both the extent and the rate of pH decline are important for pork quality (Wismer-Pedersen and Briskey, 1961; Briskey, 1964). The water-holding capacity and the color are influenced by the structural integrity of the muscle proteins. Proteins are sensitive to high temperatures and low pH values. Both conditions may cause denaturation and consequently loss of structure and function of these proteins, which gives rise to an inferior water-holding capacity and a paler color. When the final pH approaches the isoelectric point, which is for most meat proteins around 5.0 (Hamm, 1960), myofibrillar repulsion is minimized and the filament lattice shrinks. The water in muscle is captured within this lattice rather than chemically bound. A decrease in the filament lattice space, in response to a decrease in myofibrillar repulsion, results in higher drip loss formation after the meat is cut (Offer and Knight, 1988). Moreover, the same mechanism affects surface light scattering. At low final pH values more light is scattered from the meat surface, which leads to a paler color. The color of meat also depends on the concentration of myoglobin, the meat pigment, and its chemical state (Lindahl et al., 2001). The latter is especially important for the stability of the meat color during storage.

Exhaustion of animals before slaughter, due to long term stressors such as, for example, fighting after mixing of unfamiliar pigs or excessively long transport and feed withdrawal, is accompanied by the depletion of muscle glycogen (Fernandez and Tornberg, 1991). Only low amounts of lactic acid can be formed and the pH remains high throughout the postmortem period. As a consequence, the meat has a **d**ark color, **f**irm consistency, and **d**ry appearance, termed 'DFD' meat. Although more commonly observed in beef, this condition also occurs in pork. Short term stressors, on the other hand, such as rough handling immediately prior to slaughter, may hasten glycogen degradation (van der Wal et al., 1999). The resulting increase in the rate of pH decline, often accompanied by an increased muscle temperature, may result in the 'PSE' condition, that is **p**ale, **s**oft, and **e**xudative meat, which is the most frequently encountered quality defect in pork.

Objective of this thesis

The large reduction in the use of halothane-positive pigs has improved pork quality. As a consequence, very severe cases of PSE pork are much less observed in the last couple of years. Still, there remains a large variation in pork quality which gives rise to complaints by both producers and increasingly critical consumers. Pork quality may be influenced by a variety of conditions. This thesis focuses on factors that are related to processing plants. Objective is to identify pre- and postslaughter factors that are critical for the color and water-holding capacity of fresh pork. Within the individual studies, attention is paid mainly to effects on the average *level* of pork quality. In the General discussion across all studies, also the changes caused in the *variation* in color and water-holding capacity are taken into account.

In the first study (Chapter 2), pork quality was surveyed under controlled conditions at three commercial pork processing plants. This rather broad approach was narrowed down in the following experiments to the study of specific preslaughter handling aspects, namely transport and lairage (Chapter 5, 6 and Appendix to Chapter 6), as well as driving the pigs to the stunner (Chapter 3 to 6 and Appendix to Chapter 6). Electrical and CO_2 stunning could not be directly compared because the two stunning methods were employed in different processing plants. However, by standardizing other factors that may influence pork quality, also information on the effect of stunning method on pork quality was gathered (Chapter 2 and 4). Chilling was included in one of the studies as the only postmortem factor under investigation (Chapter 3). In all studies, special attention was paid to interactions between these factors in order to assess their relative importance in determining pork quality.

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Effect of processing plant on pork quality

2

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Abstract

In the present experiment the effect of processing plant on pork quality was studied by assessing pork quality in three commercial plants (A, B, C). Plants differed in the layout of the races, stunning systems (A and B: electrical, C: CO_2 stunning) and chilling systems (A: rapid chilling, B and C: conventional). Factors not related to the processing plants (e.g. genetic background of the animals, transport and lairage duration) were standardized. In total, nine groups of about 150 pigs each were purchased at commercial farms, randomly divided into three and processed on various days at the three processing plants. Muscle pH was measured in the lumbar part of the longissimus muscle (LM) at 30 min and 18 h (ultimate pH) postmortem. On a subset of animals, muscle temperature and pH were also measured at 40 min and 4 h postmortem, respectively. Pork quality was assessed in the LM by objectively (L*, a*, and b* values) and subjectively (Japanese color score) measured meat color, filter paper moisture score, electrical conductivity, and drip loss. There were no differences ($P > 0.05$) between the three plants regarding muscle temperature, 4-h and ultimate pH, lightness (L*), redness (a*), subjectively scored pork color (Japan), and drip losses after 48 h of storage. However, muscle pH at 30 min postmortem was higher ($P < 0.05$) at plant B compared with both plants A and C. Moreover, pork produced at plant B was less yellow (lower b* value; $P < 0.05$) than at plant C; pork at plant A was intermediate ($P > 0.05$). Plant C produced pork with a lower ($P < 0.05$) water-holding capacity as indicated by increased filter paper moisture scores and electrical conductivity values compared with both plant A and B. Electrical conductivity values were yet lower ($P < 0.05$) at plant A compared with plant B. It is concluded that processing plant may influence pork quality which is suggested to be related to differences in preslaughter stress level, method of stunning, and/or rate of chilling. Correlations between early postmortem measurements and meat quality traits were low. However, by grouping carcasses into temperature and pH classes, it was shown that high carcass temperatures and low pH values early postmortem were related to an inferior pork quality.

Introduction

Meat that is aberrant in color and/or water-holding properties, in its most extreme form called pale, soft and exudative meat (PSE), represents a major problem to the pork industry. Its poor processing characteristics and appearance make it unacceptable to both processors and consumers. Carcasses that develop PSE meat are characterized by an increased rate of glycolysis leading to rapid pH decline in muscle while the carcass temperature is still high (Briskey, 1964). There is a myriad of both animal-related and environmental factors that can affect muscle metabolism, pH and temperature and that predispose to the development of the PSE syndrome. Among those factors are genotype (Sellier, 1988), nutrition (Coma, 2001), feed withdrawal (Eikelenboom et al., 1991), and transport and lairage (Geverink et al., 1998). Handling and processing in slaughter plants is well-known to have a large impact on meat quality as well: Stress immediately prior to slaughter (Warriss et al., 1994; van der Wal et al., 1999), stunning method (Channon et al., 2000), and chilling rate (Offer, 1991) play important roles in the conversion of muscle to meat.

Many studies have been conducted under experimental conditions to assess the impact of the individual factors mentioned earlier. However, investigation of factors related to the processing plant imposes major problems. Small scale studies conducted under experimental conditions often fail to simulate practical conditions: Handling is usually gentler and the stress levels encountered at commercial plants, where the throughput is several hundred pigs per hour are not reached (Brown et al., 1998). Surveys involving commercial plants, on the other hand, often are not able to standardize pre-processing plant factors such as genetic background of the pigs or duration of transport and lairage (Gispert et al., 2000; Velarde et al., 2000). Another challenge in large-scale commercial studies represents the reliable identification of the carcasses on the day after slaughter when ultimate meat quality is assessed (Warriss et al., 1994).

The main objective of the present experiment was to study meat quality at three different commercial pig-processing plants on a large scale and under controlled conditions. Also, the predictive value of early-postmortem measurements for pork quality in a homogeneous pig population processed under commercial circumstances was investigated.

Materials and methods

Animals and experimental design

All pigs used in this experiment were commercial offspring of various halothane-free lines of the *Hypor* pig breeding company. Six different farms provided pigs with three of the farms providing pigs for the experiment twice. Proportion of gilts and castrated males was equal across plants. Carcass weight was 87 kg (SD 6.9) and lean percentage

measured by the Hennessy Grading probe was 56.2 (SD 2.4). A total of 1345 pigs were processed from October to March at the three plants. Nine groups of about 150 pigs each were purchased at commercial farms. Within one group, pigs came from the same farm and had the same genetic background. Each group was randomly divided into three and sent to the three processing plants on consecutive days. Not all farmers could provide 3 x 50 pigs within one week but there was always a minimum of 40 pigs per group delivered to each plant. Feed withdrawal, transport (duration and loading density), and the period between arrival at the processing plant and slaughter was standardized for each group and did not differ between plants within the same group.

Preslaughter and slaughter

Pigs from different pens were mixed and loaded early in the morning. At the processing plant experimental pigs were held separately in one pen until slaughter at 1100. This resulted in an average feed withdrawal time of about 20 h, an average transport time of 3 h 43 min and an average lairage time of 4 h per group (in Table 1 shown separately for each plant). During lairage, pigs were showered according to a standard scheme identical for all plants.

Table 1. Means ± SD for feed withdrawal, transport and lairage times at the three processing plants

	Plant A		Plant B		Plant C	
Feed withdrawal	19 h 53 min	± 72 min	20 h 17 min	± 59 min	19 h 42 min	± 71 min
Transport	3 h 35 min	± 53 min	3 h 45 min	± 50 min	3 h 49 min	± 40 min
Lairage	3 h 59 min	± 112 min	4 h 16 min	± 97 min	3 h 50 min	± 118 min

The races from the holding pens to the stunning area were different between plants: corridors narrowed down to a single-file race at plant A and to a double-file race at plants B and C. Subsequently, at both plants A and B a single-file race-restrainer with a moving belt conveyor transported the pigs to the stunning equipment. At plant C, the double-file race directly led to the stunning equipment where pigs in pairs entered the equipment alternately either from the left or the right race.

Plants A and B both used an automatic head-to-heart electrical stunning system (MIDAS, Stork RMS, the Netherlands and INARCO constant voltage system). In this system, the head electrodes are applied for 2.1 s with a minimum current of 1.3 A reached within 1.3 s. Maximum current is 2.4 A. The voltage is kept constant at 200 V. For the chest electrode that is applied 0.7 s after the head electrodes the maximum current is 0.8 A. The voltage is kept constant at 75 V.

Plant C used a carbon dioxide stunning system (COMBI 88, Butina APS, Denmark). In this so-called 'paternoster system' pigs are successively lowered into the gas: two pigs enter the equipment while at the same time two (unconscious) pigs are unloaded at the other side of the equipment. CO_2-concentration was 87%. Total time spent in the stunning equipment was about 1 min 45 sec.

Table 2. Description of the processing plants

	Plants		
	A	B	C
Races from holding pen to stunning	Single-file race followed by a single-file race-restrainer with a moving belt conveyor	Double-file race followed by a single-file race-restrainer with moving belt conveyor	Double-file race
Stunning	Electric head-to-heart	Electric head-to-heart	CO_2
Line speed (pigs/h)	500	420	500
Stunning to chilling	42 min	45 min	38 min
Chilling	3-Phase chilling tunnel (-15 / -10 / -1 °C), storage at +4 °C	Pre-chilling tunnel (3-5 °C), storage at +4 °C	Storage at +0.7-3 °C

After stunning, at plants A and B pigs were immediately exsanguinated and after the bleeding rail shackled at the right hind leg. At plant C, pigs were first shackled and then exsanguinated (bleeding occurred in the hoisted position). Line speeds were highest at plants A and C (500 pigs/h) compared with plant B (420 pigs/h). From stunning to entering the cold storage, carcasses needed 38 min at plant C, 42 min at plant A and 45 min at plant B. At plant A carcasses passed through a 3-phase-rapid chilling tunnel: section I (temp. –15 °C, air velocity (AV) 3 m/s, duration 15 min), section II (temp. –10 °C, AV 2 m/s, duration 38 min) and section III (temp. –1 °C, AV 2 m/s, duration 38 min); carcasses were stored at +4 °C (AV 0.5 m/s). At plant B carcasses passed through a pre-chilling tunnel (+3-5 °C, AV 3-3.5 m/s, duration 31 min) before reaching the cold storage (+4 °C, AV 1.5-2 m/s). Carcasses at plant C directly entered the cold storage (+0.7-3.0 °C, AV 2 m/s). Table 2 shows an overview of the main characteristics of the three plants.

Measurements

All measurements were taken in the longissimus muscle (LM) of the left carcass side at the level of the third lumbar vertebra. Measurements on a cut surface were performed

at the last lumbar vertebra. At 30 min and 18 h postmortem, pH was measured using a portable pH meter (Portamess 911 pH; Knick Elektronische Messgeräte, Berlin, Germany) equipped with a probe-type glass electrode (LoT406; Mettler Toledo, Switzerland). In three groups of pigs, pH was also measured at 4 h postmortem. On a subset of animals, temperature was measured 40 min postmortem with a portable thermometer (hand held digital thermometer; Stekon, Hoofddorp, The Netherlands). At 18 h postmortem, loins (bone-in) were collected to take the final meat quality measurements. The caudal cut surface was used to assess muscle exudate based on a method developed by Kauffman et al. (1986). After cutting, the LM muscle surface was exposed for 15 min before gently pressing a filter paper onto the surface. After 5 s, a visual score ranging from 1 (dry) to 5 (completely wet) was given (both whole-number and half-number scores). Color was both subjectively scored and objectively measured: a visual score from 1 (pale) to 6 (dark) was given by means of the Japanese color scale (Nakai et al., 1975) and the L*, a*, and b* value were assessed with a Minolta Portable Chroma Meter (Model CR 210; Minolta, Osaka, Japan) equipped with a 50-mm aperture and using illuminant D65. Electrical conductivity was measured in the LM caudal cut surface using the LF-Star (Ingenieurbüro Matthäus, Nobitz, Germany). Drip loss was measured according to a modified 'Honikel-test' by suspending a 2-cm boneless slice of the LM (lumbar region) in a net in a preserving jar for 48 h at 4 °C. The surface/thickness relation was standardized.

Statistical analysis

Data were analyzed by the mixed-model procedure (PROC MIXED) of SAS (version 8.02; SAS Inst., Inc., Cary, NC). Least squares means were generated by the LSMEANS statement. Tests of multiple comparisons of LSMEANS were adjusted according to the TUKEY-KRAMER method to ensure the overall significance level of $P = 0.05$. The model applied included the fixed effect of processing plant and the random effects of slaughter group and the two-way interaction processing plant × slaughter group which corresponds with the day of slaughter. Spearman correlation coefficients were calculated using the CORR procedure of SAS. A few data (max. 1% of all observations per trait) were discarded because they deviated by more than three times the SD from the mean. If a variable could not be measured at a plant on a specific day (e. g. due to technical problems with the equipment) that variable was excluded from statistical analysis for the entire group.

Results

Pork quality

Early postmortem temperature and pH measurements as well as ultimate pork quality attributes are shown in Table 3. At 30 min postmortem, pH was higher ($P < 0.05$) at plant B compared with both plant A and C. Although plant B at 4 h postmortem still

produced numerically the highest pH, differences were statistically not different anymore (P = 0.15). Ultimate pH, measured 18 h postmortem, was not different between the three plants.

Temperature, which was measured on a subset of animals, was with 40.3°C numerically highest at plant B, followed by plant C (39.7°C) and plant A (39.1°C) but differences were not significant (P = 0.14).

Table 3. Effect of processing plant on pH, temperature, and pork quality attributes in the longissimus muscle[a]

	N			LSmeans				
Plant:	A	B	C	A	B	C	Pooled SE	*P*-value
pH								
30 min	364	359	371	6.49[x]	6.70[y]	6.52[x]	0.046	0.001
4 h[b]	142	144	164	6.06	6.13	5.98	0.058	0.150
18 h (ultimate)	377	399	393	5.72	5.68	5.61	0.05	0.369
40-min temperature, °C[b]	87	99	97	39.1	40.3	39.7	0.29	0.138
L*[c]	329	349	352	49.9	49.5	50.1	0.40	0.496
a*[c]	329	349	352	17.2	17.1	17.0	0.25	0.781
b*[c]	329	349	352	7.5[xy]	7.1[x]	7.6[y]	0.16	0.011
Japan[d]	371	398	393	2.9	3.0	2.7	0.10	0.172
FPS[e]	370	357	393	2.5[x]	2.8[x]	3.3[y]	0.17	0.001
EC, mS[f]	377	401	397	5.4[x]	6.4[y]	7.4[z]	0.36	0.001
48-h drip loss, %[g]	330	352	354	4.8	4.9	5.2	0.36	0.474

[a]Data in the table are presented as least squares means.
[b]Muscle pH at 4 h and muscle temperature at 40 min postmortem were measured in a subset of animals.
[c]L* = a measure of darkness-lightness (higher value indicates a lighter color); a* = a measure of redness (higher value indicates a redder color); and b* = a measure of yellowness (higher value indicates a more yellow color).
[d]Japan = color score on a scale from 1 (light) to 6 (dark).
[e]FPS = filter-paper measured moisture content (score from 1 to 5; higher scores indicate a higher moisture content and a lower water-holding capacity).
[f]EC = electrical conductivity (a higher value indicates a lower water-holding capacity).
[g]Drip loss percentages were calculated after storage at 4°C for 48 h.
[xyz]Least squares means lacking a common superscript letter differ (P < 0.05).

Pork was less yellow (b* value; P < 0.05) at plant B compared with plant C. Pork at plant A exhibited a similar yellowness as pork produced at plant B or plant C.

Lightness (L* value), redness (a* value) and the Japanese color score, on the other hand, were similar at all plants.

All plants produced pork with a similar amount of drip loss developing during 48 h of storage. However, pork produced at plant C had a higher ($P < 0.05$) filter paper moisture score than pork produced at both plants A and B. Also, electrical conductivity values were highest ($P < 0.05$) at plant C, pointing, similar to the filter paper moisture score, to a lower water-holding capacity. Conductivity values for pork produced at plant A were yet lower ($P < 0.05$) than values registered at plant B.

Table 4. Spearman correlation coefficients

	pH_{4h}	pH_{18h}	T_{40min}	L*	Japan	FPS	EC	Drip[h]
pH_{30min}[a]	0.37	0.15	-0.16	-0.12	0.17	-0.31	-0.39	-0.31
pH_{4h}[ab]		0.21	-0.19	-0.23	0.20	-0.29	-0.48	-0.43
pH_{18h}[a]			-0.19	-0.33	0.21	-0.39	-0.35	-0.39
T_{40min}[bc]				ns	ns	0.28	0.41	0.23
L*[d]					-0.55	0.38	0.24	0.44
Japan[e]						-0.31	-0.21	-0.40
FPS[f]							0.65	0.63
EC[g]								0.59

[a]Muscle pH measured in the longissimus muscle at 30 min, 4 h, and 18 h (ultimate pH) postmortem.
[b]Muscle pH at 4 h and muscle temperature at 40 min postmortem were measured in a subset of animals.
[c]Muscle temperature measured in the longissimus muscle at 40 min postmortem
[d]L* = a measure of darkness-lightness (higher value indicates a lighter color)
[e]Japan = color score on a scale from 1 (light) to 6 (dark).
[f]FPS = filter-paper measured moisture content (score from 1 to 5; higher scores indicate a higher moisture content and a lower water-holding capacity).
[g]EC = electrical conductivity (a higher value indicates a lower water-holding capacity).
[h]Drip loss percentages were calculated after storage at 4°C for 48 h.
ns = not significant ($P > 0.05$).

Correlations between pork quality attributes

Table 4 shows that correlations between early postmortem pH and temperature and pork quality attributes were low to moderately high. None of the three pH measurements (at 30 min, 4 h, and 18 h postmortem, respectively) showed high correlations with other final pork quality attributes. Correlations between early postmortem muscle temperature and color measurements were not significant ($P > 0.05$) whereas the correlation between 40-min muscle temperature and electrical

conductivity was moderate (r = 0.41). Color, both subjectively scored and instrumentally measured, had low to moderately high correlations with traits related to water-holding properties (filter paper moisture, electrical conductivity, drip losses). Lightness (L* value) showed higher correlations than redness (a* value) or yellowness (b* value), which are not shown. Highest correlations were found between traits related to water-holding properties.

Discussion

Pork quality regarding pH, temperature, and subjectively scored color (Japan) was similar to values obtained in the studies of van der Wal et al. (1997, 1999). The main objective of this work was to assess the impact of processing plants on the quality of pork and indeed, although the differences were small, meat quality varied between the three plants: pork processed at plant C had slightly inferior water-holding properties than pork processed at either plant A or B. The experimental design aimed at eliminating effects related to genetics, feed or other preslaughter factors such as transport and lairage. So, largely factors associated with the processing plants must be responsible for the differences in pork quality. The level of preslaughter stress, stunning, and chilling systems were the most important differences between the plants and will be discussed in reverse order. It has to be kept in mind, however, that these factors were confounded between plants which makes it difficult to draw firm conclusions regarding the causal relationships between the above factors and the observed differences in pork quality between the processing plants.

Chilling

All three chilling systems were different: Highest chilling rates were achieved at plant A where carcasses passed through a rapid chilling tunnel whereas chilling rates were lowest at plant B. Offer (1991) modeled the formation of PSE meat and showed that especially at low rates of pH fall increasing the chilling rate is very effective in reducing myosin denaturation and thus drip losses. In contrast, studies by Gigiel et al. (1989) and van der Wal et al. (1995a) indicate that different chilling rates do not affect meat quality. In view of these studies, chilling rate is believed to be of minor importance regarding the observed differences in meat quality between the plants.

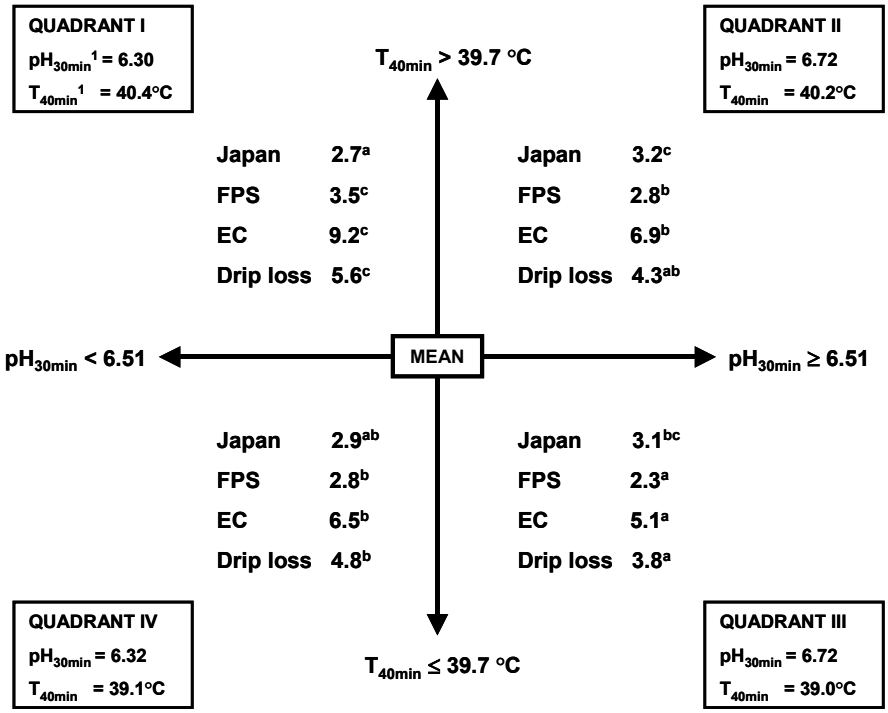
Stunning method

Another possible source for the observed variation in pork quality may relate to the stunning systems: Plants A and B operated an electrical stunning system compared with plant C which used a CO_2 stunning unit. In this context it is interesting that plant C produced the worst meat quality. In contrast to our results, several studies report a positive effect on meat quality of CO_2 stunning compared with electrical stunning (Henckel et al., 1998; Channon et al., 2000; Velarde et al., 2000, 2001). However, in some of these studies, stress-sensitive genotypes were used, which is important: In the study

of Channon et al. (2000), the positive effect of CO_2 stunning was clearly seen with the stress-sensitive animals but differences between CO_2 and electrical stunning disappeared when stress-resistant genotypes were carefully handled. Thus, the effect of stunning method depends on genotype and the circumstances under which it is assessed. Another reason for the discrepancies may relate to differences in stunning equipments. In the studies of Henckel et al. (1998) and Channon et al. (2000) where electrical stunning produced inferior pork compared with CO_2 stunning, electrical stunning was applied manually whereas in the present study fully automated electrical head-to-heart stunning equipment was used. However, differences in stunning equipment do not explain results found by Velarde et al. (2000, 2001) since the two stunning systems (CO_2 and electrical) closely resembled the ones used in the present experiment. They found, in both studies, higher incidences of PSE meat in processing plants employing electrical stunning, as opposed to the plants where CO_2 stunning was used. However, in both studies the plants equipped with electrical stunning systems had considerably higher slaughter line speeds compared with the plants using CO_2 stunning. As Warriss et al. (1994) showed, higher slaughter line speeds negatively affect meat quality and this may have confounded the results. The present study suggests that CO_2 stunning is not necessarily a guarantee for a better pork quality in terms of color and water-holding properties as suggested by other authors.

Preslaughter stress

Beside contrasts between the three processing plants in chilling and stunning regimes there remains another important cause for variation in pork quality: level of pre-slaughter stress. Van der Wal et al. (1997) investigated factors influencing pork quality and concluded that the period immediately before stunning may have a large effect on pork quality. In a more specific experiment, van der Wal et al. (1999) could show that the application of 1 min of stress directly before stunning decreased muscle pH at 45 min (6.39 vs. 6.66), increased muscle temperature at that time (39.9 vs. 39.4 ^{o}C) and negatively affected water-holding capacity (51.2 vs. 30.6 mg filter paper wetness) for gilts. In the present experiment stress was not deliberately inflicted on the pigs. However, stress inevitably accompanies the slaughter process (Brown et al., 1998). Grandin (1996) identified a myriad of factors such as aspects of lighting and ventilation, noises or floor conditions that impede animal movement and may cause excitement and stress to slaughter pigs. Moreover, Warriss et al. (1992) showed that the width of races and bends influences the ease and speed with which pigs move towards the stunning area. To sum up, although preslaughter conditions in the present experiment were standardized as far as possible, the period immediately prior to stunning was somewhat different between plants since the personnel and design of the races differed. Thus, higher preslaughter stress probably has contributed to the slightly lower quality of pork produced at plant C; however, stress levels at the individual plants were not assessed to support this hypothesis.



EC **= electrical conductivity;** FPS **= filter paper moisture score;** Drip **loss = 48-h drip loss;** Japan **= Japanese color score**

[1]Means for the respective group

Figure 1. Means for selected pork quality attributes grouped according to early postmortem temperature (T_{40min}) and pH (pH_{30min}). Samples with T_{40min} and pH_{30min} below or above the mean of all samples (T_{40min} = 39.7°C, pH_{30min} = 6.51) are combined in the same quadrant. Means in different quadrants lacking a common superscript letter differ ($P < 0.05$).

Prediction of meat quality by pH and temperature early postmortem

Correlations between early postmortem pH and temperature and pork quality traits such as drip loss or color were low but in line with other studies performed under commercial circumstances (Kauffman et al., 1993; van der Wal et al., 1995b). In these studies, pH proved to be the most valuable single predictor of meat quality traits such as drip loss but, as in the present study, meat quality could not be reliably predicted by any single or combined early post-mortem measurement. On the other hand, Schäfer et al. (2002) showed that pH and temperature early post-mortem explained up to 89 % of variation in drip loss observed. In their study two pre-slaughter stress treatments were

used to create large variation in drip loss which simultaneously led to a large variations in early postmortem pH and temperature. Within the more limited range for these variables in the present study and under less than optimal conditions for taking measurements (commercial processing plant vs. experimental abattoir), no such high correlations could be found. Yet early postmortem temperature and pH clearly affected pork quality. To illustrate this, carcasses were grouped according to whether they showed a higher or lower temperature and pH early postmortem compared to the mean of all carcasses. This resulted in four groups of 92-132 samples each differing in their early postmortem pH and temperature values. These were assigned to four quadrants formed by a horizontal and a vertical axis representing pH and temperature, respectively (Figure 1). Subsequently, pork quality traits of the four groups were statistically analyzed. Carcasses exhibiting a higher than average carcass temperature and a lower than average pH early postmortem (samples in the upper-left quadrant) gave an inferior meat quality than the other groups. The best quality was encountered in samples located in the opposite (lower-right) quadrant characterized by a lower than average carcass temperature and a higher than average pH. Samples having either less favorable temperature *or* less favorable pH values ranked in between. Thus, although final pork quality could not be predicted by pH or temperature, it could be shown that it is important to prevent conditions where pig carcasses develop a high temperature and a low muscle pH early postmortem.

Implications

Comparison between three plants revealed that processing plant may cause variation in pork quality independent from preslaughter factors such as genetic background of the animals or transport and lairage. Since factors such as stress level, stunning method, and chilling rate were confounded, no causal relationships could be established. Future work should aim at unraveling those factors causing variation in pork quality at commercial processing plants. Although it was not possible to reliably predict final meat quality in the homogeneous pig population used in this experiment, it was shown that high carcass temperatures and low pH values early postmortem lead to inferior pork quality.

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Rapid chilling cannot prevent inferior pork quality caused by high preslaughter stress

3

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Abstract

The present experiment investigated whether increasing chilling rate could improve meat quality in pigs exposed to either minimal or high stress immediately preslaughter. Pigs (n = 192) were offspring of halothane-free lines. On various days, four groups of 48 pigs were processed at a commercial plant. Within each group, half the pigs were exposed to either minimal or high preslaughter stress. Before entering the cooler at 45 min postmortem, carcasses of both minimal and high preslaughter stress treatments were allocated randomly to either conventional (+4 °C for 22 h) or rapid (three-phase chilling tunnel: -15, -10, and -1°C for 15, 38, and 38 min, respectively, followed by storage at 4°C until 22 h postmortem) chilling. Temperature and pH were measured in the blood at exsanguination and in the longissimus muscle (LM) and semimembranosus muscle (SM) at 0.5, 2.5, 4.5, 6.5, and 22 h postmortem. Meat quality attributes (water-holding capacity and objective color measurements) were assessed on the LM. Preslaughter stress level affected pH and temperature in both blood and muscle, with lower ($P < 0.001$) pH values, and higher ($P < 0.001$) temperatures for pigs exposed to high vs. minimal stress. Rapid chilling led to a faster ($P < 0.001$) temperature decline regardless of preslaughter stress level. Rapid chilling did not ($P > 0.05$) influence the rate of pH decline in the LM muscle, but reduced ($P = 0.061$) pH decline in the SM. Rapid chilling, as opposed to conventional chilling, decreased ($P < 0.05$) electrical conductivity in the LM, regardless of preslaughter stress; however, it could not compensate for the detrimental effect ($P < 0.05$) of stress on drip loss, filter paper moisture absorption, and meat color (L* value). Results from the present study indicated that increasing chilling rate is not a suitable method to resolve pork quality problems caused by inadequate preslaughter handling.

Introduction

Pale color and high drip losses, two common problems in pork, are pH- and temperature-dependent phenomena. Exposure to high temperatures at a low pH causes denaturation of muscle proteins, which impairs water-holding capacity and meat color (Wismer-Pedersen, 1959; van der Wal and Eikelenboom, 1984; Offer and Knight, 1988). There are various factors that can increase the rate of decline in pH and carcass temperature. Stress in the immediate preslaughter period is one important factor, playing a crucial role even with stress-resistant breeds (Warriss et al., 1994; van der Wal et al., 1999; Channon et al., 2000). Prevention of stressful events prior to slaughter is often difficult, and additional strategies to lessen the impact of preslaughter stress are needed.

Increasing chilling rate decreased drip loss and/or improved meat color in some studies (Kerth et al., 2001; Milligan et al., 1998) but not in others (Gigiel et al., 1989, Long and Tarrant, 1990; van der Wal et al., 1995). Meade and Miller (1990) reported a higher pH for highly stressed pigs that were hot-fat trimmed, facilitating a more rapid temperature decline in the carcass compared with highly stressed pigs that were not hot-fat trimmed; however, there was no difference observed between hot-fat and non-hot-fat-trimmed carcasses of unstressed pigs. Likewise, Kerth et al. (2001) observed a reduction in PSE meat in the loins and hams of stress-sensitive pigs after accelerated chilling but not in stress-resistant pigs. Based on these studies, it was hypothesized that increasing the rate of temperature decline to improve meat quality would be particularly effective in highly stressed pigs. Therefore, the aim of the present experiment was to compare the effect of two commercial chilling methods (conventional vs. rapid) on meat quality (color and drip loss) of pigs after either minimal or high preslaughter stress.

Materials and methods

The experimental protocol was approved by the Animal Care and Ethics Committee of the University of Nijmegen, The Netherlands.

Animals and experimental design

All pigs were commercial, halothane-free crossbreeds (gilts and barrows) with an average hot carcass weight of 89.8 kg and a lean percentage of 55.9% measured by the Hennessey Grading Probe. There were no differences ($P > 0.05$) in carcass weight or lean percentage between treatments. In a completely randomized design, pigs (n = 192) were assigned to one of four treatments in a 2 × 2 factorial arrangement, with two preslaughter stress levels (minimal or high preslaughter stress) and two chilling methods (conventional or rapid chilling). Every slaughter group originated from a

different commercial farm. Four groups of 48 pigs (12 pigs per treatment combination) were used, resulting in a total of 48 pigs per treatment.

Preslaughter and slaughter

All farms were located within close distance to the processing plant (< 20 km). After transport, pigs were held in lairage for 3 to 8 h and slaughtered at 0630. Experimental stress treatments started approximately 5 min before slaughter. Pigs of the minimal stress group were guided to the stunning area without the use of electric goads and were handled as calmly as possible. Pigs of the high stress group were forced by yells and electric goads to move four times back and forth in the corridor leading to the stunning area. Unpublished data from our laboratory has shown that this high-stress treatment led to an almost twofold increase in blood lactate levels and substantially higher blood cortisol levels when compared to the minimal stress treatment. Pigs were electrically stunned in a fully automated head-to-heart stunning system (MIDAS, Stork, The Netherlands).

Chilling

Shortly before entering the cold storage (45 min postmortem), pigs within the same preslaughter stress treatment were randomly allocated in groups of six to either the conventional or the rapid chilling system. Conventionally chilled carcasses entered the cooler and were kept at 4°C (air velocity of 0.5 m/s) until 22 h postmortem. Rapidly chilled carcasses passed through a three-phase chilling tunnel: 1) -15°C for 15 min (air velocity of 3 m/s); 2) -10 °C for 38 min (air velocity of 2 m/s); and 3) -1°C for 38 min (air velocity of 2 m/s). After passing through the chilling tunnel, carcasses were held at 4°C until 22 h postmortem, similar to the manner in which conventionally chilled carcasses were handled.

Measurements

At exsanguination, pH and temperature were measured in blood using a portable pH meter (Portamess 911 pH; Knick Elektronische Messgeräte, Berlin, Germany) equipped with a probe-type glass electrode (LoT406; Mettler Toledo, Switzerland) and a portable thermometer (hand held digital thermometer; Stekon, Hoofddorp, The Netherlands). In muscle, pH and temperature were measured in the longissimus muscle (LM) at the level of the third lumbar vertebra and in the semimembranosus muscle (SM). Muscle pH and temperature in the SM were measured with the same instruments at 0.5, 2.5, 4.5, and 6.5 h, and pH was also measured at 22 h postmortem. In the LM, temperature was recorded at 5-min intervals from 0.5 to 22 h postmortem with a data logger (Diligence EV N2002; Comark Instruments, Stevenage, U.K.) equipped with a food penetration probe (PX22L/C; Comark Instruments). Only data corresponding with the measurement times in the SM are presented (0.5, 2.5, 4.5, 6.5, and 22 h postmortem). The day after slaughter (22 h postmortem), final meat quality measurements were

taken in the same region of the LM as muscle pH and temperature (third lumbar vertebra). Internal light scattering was measured using the fiber optic probe (TBL Fibres, Leeds, U.K.). Meat color was determined after a 10-min blooming period by measuring the L*, a*, and b* values with a Minolta Portable Chroma Meter (model CR 210; Osaka, Japan) equipped with a 50-mm aperture and using illuminant D65. Electrical conductivity was measured using the LF-Star (Ingenieurbüro Matthäus, Nobitz, Germany).

Water-holding capacity of the LM was measured by two different methods. A filter paper (45-mm diameter) was weighed, gently pressed on the caudal cut surface of the LM for 10 s, and subsequently reweighed to determine the absorbed moisture content. Additionally, samples of the LM were placed with a cut surface facing down on a metal grid that was placed in a closed plastic container. Drip loss was determined as percentage of weight loss after 1 and 2 days of storage at 4°C.

Statistical analysis

Data were analyzed by the mixed-model procedure (PROC MIXED) of SAS (version 8.02; SAS Inst., Inc., Cary, NC). Least squares means were generated by the LSMEANS statement. Tests of multiple comparisons of LSMEANS were adjusted according to the TUKEY-KRAMER method to ensure the overall significance level of $P = 0.05$. The model applied for the pH and temperature measurements in the LM and the SM included the fixed effects of stress level and chilling method, their interaction, the random effect of slaughter day, and the repeated effect of time with pig as subject. The model applied for pH and temperature measured in blood, as well as for all pork quality attributes measured in the LM, included the fixed effects of stress level, chilling method, their two-way interaction, and the random effect of slaughter day.

Results

Temperature decline

Temperature declines in the LM and in the SM muscle are shown in Figure 1. Both stress level and chilling method affected ($P < 0.001$) the time course of temperature decline, with a (numerically) greater effect of chilling method as opposed to stress level. At exsanguination, highly stressed pigs had a 0.3°C higher ($P < 0.05$) blood temperature than minimally-stressed pigs (40.0 vs. 39.7°C). At 0.5 h postmortem, the temperature difference had increased ($P < 0.05$) to about 1°C for both the LM and the SM muscles. Temperatures for the LM from the highly and minimally stressed groups were 40.9 and 39.8°C, respectively, whereas SM temperatures were 42.3 and 41.4°C in pigs subjected to high and minimal levels of stress, respectively. In the LM, the difference between the two stress levels persisted until 6.5 h postmortem even though pair-wise comparisons did not reveal statistically significant differences ($P > 0.05$). In the SM, there was no temperature difference between high and minimal stress for rapidly chilled carcasses,

whereas high stress in combination with conventional chilling led to a higher ($P < 0.05$) temperature than the minimal stress and conventional chilling treatment combination at 6.5 h postmortem (stress level × chilling method interaction; $P = 0.015$).

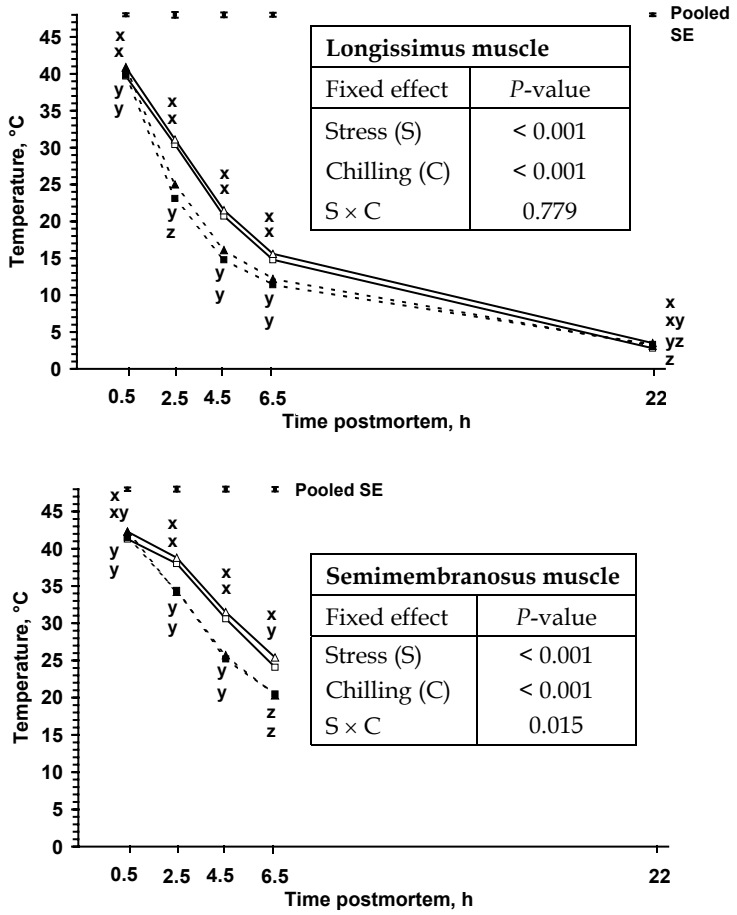

Figure 1. Temperature decline in the longissimus and semimembranosus muscles. Each data point represents the least squares means of 45 to 48 carcasses, whereas the pooled SE is represented by the error bars at the top of each graph. Within a specific time postmortem, least squares means that do not have a common letter differ ($P < 0.05$).

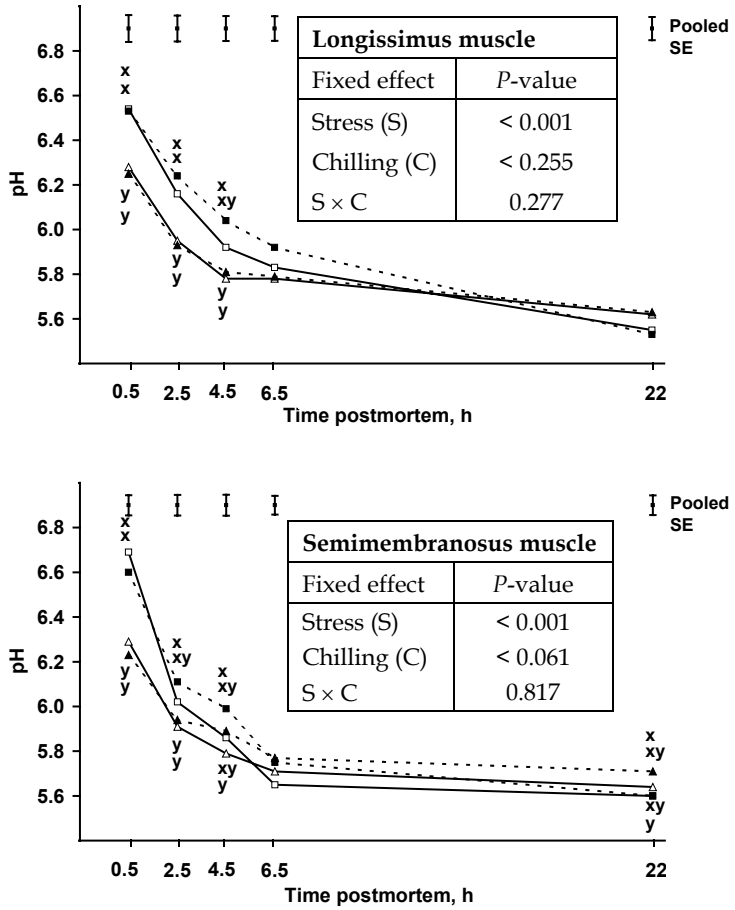

Figure 2. pH decline in the the longissimus lumborum and semimembranosus muscles. Each data point represents the least squares means of 45 to 48 carcasses, whereas the pooled SE is represented by the error bars at the top of each graph. Within a specific time postmortem, least squares means that do not have a common letter differ ($P < 0.05$).

pH Decline

Measurements of pH in blood and in the LM and SM muscles are presented in Figure 2. Pigs of the high stress group had a lower ($P < 0.05$) blood pH at exsanguination than pigs in the minimal stress group (7.03 vs. 7.16, respectively). At 0.5 h postmortem, a greater contrast in pH was measured in both muscles ($P < 0.05$); in the LM, high stress led to a pH of 6.27 compared to 6.54 for the minimal stress pigs; similarly, in the SM, highly stressed pigs had a pH of 6.26 vs. 6.68 for minimally stressed pigs. The subsequent chilling did not ($P = 0.255$) affect pH decline in the LM, whereas rapid chilling led to a slower ($P = 0.061$) pH decline in the SM. Ultimate (22-h) pH was similar ($P > 0.05$) for most treatments (5.53 to 5.63 in the LM and 5.60 to 5.71 in the SM). Only in the SM, pair-wise comparisons revealed a difference ($P < 0.05$) between rapidly chilled carcasses of the high stress treatment (pH 5.71) and conventionally chilled carcasses of the minimal stress treatment (pH 5.60).

Meat quality

Results of the meat quality measurements are presented in Table 1. High preslaughter stress increased ($P < 0.05$) internal light scattering, L* values, electrical conductivity, filter paper moisture, and both 24- and 48-h drip loss percentages compared with minimal preslaughter stress. Rapid, compared with conventional, chilling increased ($P < 0.05$) internal light scattering and decreased ($P < 0.05$) electrical conductivity.

Discussion

High preslaughter stress has been shown to increase carcass temperature and the rate of pH decline (Warriss et al., 1994; van der Wal et al., 1999; Channon et al., 2000). These effects were also observed in the present study. However, the main objective of the present experiment was to study the combined effects of preslaughter stress and two different chilling systems on meat quality rather than just the effects of preslaughter stress on its own.

Differences in final meat quality were mainly attributable to the effect of preslaughter stress (Figure 3). High preslaughter stress led to an approximately 50% increase in drip loss, filter paper moisture, and electrical conductivity compared with minimally stressed pigs. Conversely, rapid chilling could improve electrical conductivity by no more than 10%. Fiberoptic-measured light scattering within the LM was the exception because it was impaired rather than improved by rapid chilling. This latter effect is not confirmed by other studies testing systems with an even higher chilling rate (Gigiel and James, 1984; Long and Tarrant, 1990). In the present study, meat color was, to a lesser extent, affected by preslaughter stress than water-holding properties, whereas chilling system did not affect meat color. According to Offer and Knight (1988), alterations in initial pH hardly affect light scattering when the initial pH is relatively high (45-min pH > 6.1). Conversely, the same authors state that alterations in initial pH in those

higher ranges affect drip losses. In the present experiment, initial pH (measured at 30 min postmortem) was well above the limit of 6.1, regardless of stress level. This may explain the observation that preslaughter stress affected water-holding properties more than meat color. However, it does not explain why the (albeit small) positive effect of rapid chilling on electrical conductivity, a trait related to water-holding properties, for both high and minimal stress did not correspond with a concomitant effect on pH decline in the LM muscle.

Table 1. Effect of chilling method (C) and preslaughter stress level (S) on pork quality attributes of the longissimus lumborum muscle[a]

	Chilling method							
	Conventional		Rapid			*P*-value		
Stress level:	Minimal	High	Minimal	High	Pooled SE	S	C	S × C
n[b]	47	45	45	48				
FOP[c]	33.8	40.3	40.1	46.9	3.35	0.001	0.002	0.951
L*[d]	50.9	52.4	51.4	52.2	1.11	0.025	0.741	0.489
a*[d]	17.8	17.7	17.5	17.9	0.20	0.350	0.614	0.066
b*[d]	6.4	6.3	6.4	6.4	0.21	0.626	0.555	0.618
EC[e], mS	5.9	9.2	5.3	8.4	0.61	0.001	0.026	0.693
FPW[f], mg	89	130	80	127	8.0	0.001	0.295	0.589
Drip loss[g], %								
24 h	1.36	1.90	1.11	2.16	0.21	0.001	0.972	0.056
48 h	2.27	2.96	1.98	3.35	0.31	0.001	0.794	0.070

[a]Data in the table are presented as least squares means.
[b]For meat color measurements (L*, a*, b*) n = 36, 33, 33, and 36/treatment, respectively.
[c]FOP = fiber optic-measured light scattering (higher value is indicative of greater light scattering and paler color).
[d]L* = a measure of darkness-lightness (higher value indicates a lighter color); a* = a measure of redness (higher value indicates a redder color); and b* = a measure of yellowness (higher value indicates a more yellow color).
[e]EC = electrical conductivity (a higher value indicates a lower water-holding capacity).
[f]FPW = filter-paper measured moisture content.
[g]Drip loss percentages were calculated after storage at 4°C for either 24 or 48 h.

Jones et al. (1993) reported that blast chilling did not decrease protein denaturation. Nevertheless, in contrast to the results of the present study, Jones et al. (1993) and Milligan et al. (1998) reported a darker muscle color after blast chilling. Muscle color may not only be influenced by protein denaturation but also by the absorption characteristics of myoglobin, as well as reduced light scattering of the meat surface,

typically associated with high ultimate pH values (Lawrie, 1998). Indeed, in both experiments (Jones et al., 1993; Milligan et al., 1998), the blast chilling treatments were associated with a higher ultimate pH compared to the conventional chilling treatments. In the present study, ultimate pH in the loin was not affected by chilling system, and in agreement, no effect on color was observed.

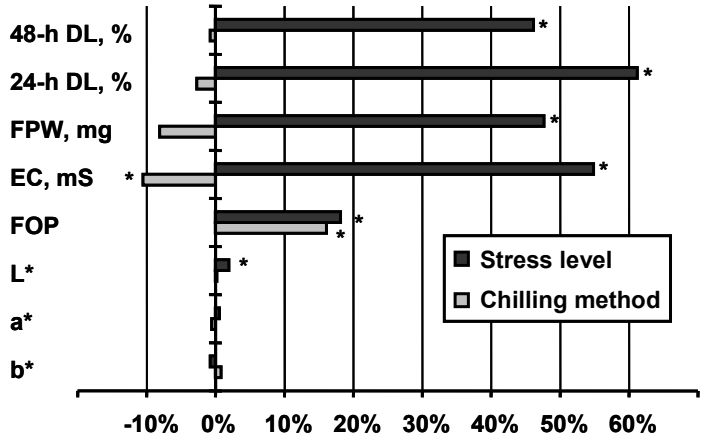

Figure 3. Percentage of change in pork quality attributes between preslaughter stress levels (minimal vs. high) and chilling method (conventional vs. rapid). Asterisks associated with a bar indicate differences ($P < 0.05$) between stress levels or chilling methods (DL = drip loss percentage after storage for either 24 or 48 h; FPW = filter-paper measured moisture; EC = electrical conductivity; and FOP = fiber optic-measured light scattering. Also, L* = a measure of lightness; a* = a measure of redness; and b* = a measure of yellowness).

Although increasing the rate of chilling leads to a more rapid temperature decline in the carcass and often to a slower pH decline (Long and Tarrant, 1990; Jones et al., 1993; Milligan et al., 1998), chilling does not necessarily induce a significant decrease in protein denaturation. This may be due to the fact that chilling affects pH mainly in the period between 3 to 4 hours postmortem (Long and Tarrant, 1990; Milligan et al., 1998), which is in accordance with the results of the present study. It is, however, the period immediately postmortem, when carcass temperatures are above 30°C, that is most critical. If muscle pH is low during the first hour postmortem, protein denaturation occurs with negative consequences for meat quality (Wismer-Pedersen, 1959; van der Wal and Eikelenboom, 1984; Offer, 1991). It is thus postulated that chilling starts too late to repair damage that has been caused at an earlier stage in the pork chain. According to Offer (1991), this is especially true in carcasses with a very rapid pH

decline because, in extreme cases, these carcasses may have completed postmortem glycolysis before entering the cooler. This was not true even for the highly stressed pigs in the present study; however, our initial hypothesis that increasing chilling rate would be particularly effective in carcasses of previously stressed pigs could not be confirmed by the outcomes of the present study.

Using higher chilling rates than those used in the present experiment may increase the effect of chilling on meat quality. However, Gigiel and James (1984), Gigiel et al. (1989), and van der Wal et al. (1995) studied various chilling systems operating at temperatures between −20 and −40°C and failed to note any effects on meat color or water-holding capacity. Moreover, it is emphasized that increasing chilling rate is not likely to fully overcome the detrimental effects of preslaughter stress.

Implications

The rapid chilling system applied in the present study could not compensate for the detrimental effect that preslaughter stress had on meat color and water-holding capacity of fresh pork. Even though increasing the chilling rate may have produced greater effects on pork quality, benefits are expected to be limited because severe preslaughter stress is very likely to have a greater effect than chilling rate. Moreover, the greatest damage likely occurs during the inevitable time lag between slaughter and start of chilling when carcass temperatures are highest. Investing in methods that help reduce stress in the immediate preslaughter period is recommended.

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Preslaughter stress and muscle energy largely determine pork quality at two commercial processing plants

4

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Abstract

The objective of the present experiment was to study physiological changes elicited in response to stress in the immediate preslaughter period and to link them to pork quality characteristics. Crossbred, halothane-free pigs (n = 192) were processed in eight groups (24 pigs per group) on various days at one of two commercial processing plants operating different stunning systems (electrical and CO_2 stunning in plants A and B, respectively). In each group, half the pigs were exposed to either minimal or high preslaughter stress. Blood samples were taken at exsanguination, and lactate, cortisol, and catecholamines, as well as blood pH and temperature, were assessed and linked to various longissimus muscle quality attributes. Additionally, muscle pH and temperature were measured 30 min postmortem, and muscle glycolytic potential was determined 22 h postmortem. At both processing plants, high preslaughter stress resulted in higher ($P < 0.05$) blood cortisol and lactate; however, the effects of preslaughter stress on catecholamines and blood pH were believed to be biased by the different stunning methods employed at the plants. High preslaughter stress increased ($P < 0.05$) blood temperature at plant A but not at plant B. At both plants, high stress increased ($P < 0.05$) 30-min muscle temperature and decreased ($P < 0.05$) 30-min muscle pH. Ultimate pH was increased ($P < 0.05$) and muscle glycolytic potential was decreased ($P < 0.05$) by high preslaughter stress. At both plants, high stress resulted in inferior pork quality attributes ($P < 0.05$), including reflectance, electrical conductivity, filter paper moisture, drip loss, and L* value. The effect of stress was greater on water-holding capacity than on pork color, with drip losses increased by 56%. Of all stress indicators measured at exsanguination, only blood lactate was strongly correlated with pork quality attributes. Regression analyses revealed that blood lactate and glycolytic potential accounted for 52 and 48% of the variation in drip loss and L* value, respectively. In combination with high preslaughter stress, high glycolytic potentials were related to increased drip losses. It is concluded that high preslaughter stress leads to impaired pork quality, with high muscle energy levels aggravating the negative effects of preslaughter stress. Monitoring stress level by blood lactate measurement in combination with strategies to control muscle energy present at slaughter may help to improve meat quality.

Introduction

Pale, soft, and exudative (PSE) pork and red, soft, and exudative pork cause economical losses for the pork industry. It is widely accepted that stress immediately before slaughter plays a crucial role, even with stress-resistant breeds (Warriss et al., 1994; van der Wal et al., 1999; Channon et al., 2000). Stress may increase the temperature and rate of pH decline in the carcass early postmortem, resulting in characteristic PSE pork (Briskey, 1964; Offer and Knight, 1988). However, pH and temperature measured at 30 to 45 min postmortem do not sufficiently explain variation in meat quality (Kauffman et al., 1993; van der Wal et al., 1995b; Hambrecht et al., 2003).

Seldom a direct causal relationship has been established between the stress level of individual pigs and the resulting meat quality. Most experiments studying preslaughter stress have either not considered meat quality (Troeger, 1989; Weeding et al., 1993; Hartung et al., 1997) or have not measured variables directly related to stress (van der Wal et al., 1999; Channon et al., 2000). Warriss et al. (1998) found low, or no, correlations between selected blood-based stress indicators and meat quality in a survey under commercial circumstances. In that study, however, no further information on the handling history nor on the background of the pigs was taken into account. Therefore, the aim of this study was to establish a link between pork quality and various physiological variables and to select the variables that show potential to serve as monitoring tools for controlling preslaughter stress levels.

Materials and methods

The experimental protocol was approved by the Animal Care and Ethics Committee of the University of Nijmegen, The Netherlands.

Animals and experimental design

All pigs were commercial, halothane-free crossbreeds with an average hot carcass weight of 89.4 kg and a lean percentage of 55.5 measured by the Hennessey Grading Probe. Gilts and barrows were equally distributed across treatments and randomly assigned to either minimal or high preslaughter stress before transportation to one of two commercial pork processing plants (A or B). Four groups (24 pigs per group) were used, resulting in 48 pigs/treatment at each plant. Every slaughter group originated from a different commercial farm and was processed either a Plant A or Plant B.

Preslaughter and slaughter

All farms were located near (< 35 km) the respective processing plant. Depending on time of arrival, pigs were held in lairage for 3 to 8 hours before slaughter at 0630. Experimental stressor treatments were begun approximately 5 min before slaughter. Pigs in the minimal stress group were guided to the stunning area without the use of

electric goads and were handled as calmly as possible, whereas pigs in the high preslaughter stress group were forced, by yells and electric goads, to move four times back and forth in the corridor leading to the stunning area. At Plant A, pigs were electrically stunned in a fully automated, head-to-heart stunning system (MIDAS, Stork, The Netherlands), whereas at Plant B pigs were stunned by carbon dioxide (COMBI88, Butina, Denmark). Carcass dressing and fabrication processes were similar at both plants.

Sampling procedures and chemical analyses

At exsanguination, from each pig two blood samples (2 × 9 mL) were collected in heparinized tubes (Monovette LH; Sarstedt, Nümbrecht, Germany) for cortisol and lactate determination, or collected in tubes containing EDTA (Monovette EDTA KE; Sarstedt) for catecholamine determination. Blood samples were immediately put on ice and centrifuged for 10 min at 1,300 × *g* within 1 h after sampling. Plasma (0.5 mL) was transferred to Eppendorf tubes, and stored at -80°C until analysis. Plasma cortisol concentrations were determined using a solid-phase RIA kit (Coat-a-Count Cortisol TKCO; Diagnostic Products Corporation, Apeldoorn, The Netherlands). Plasma lactate concentrations were determined using a blood analyzer (ABL 605; Radiometer Nederland BV, Zoetermeer, The Netherlands). Additional plasma samples were analyzed for epinephrine and norepinephrine by HPLC with electrochemical detection procedure described by Smedes et al. (1982).

For determination of the glycolytic potential, muscle samples were taken from the longissimus muscle at the fourth lumbar vertebra, rapidly put on dry ice, and stored at -80°C until analysis. According to Maribo et al. (1999), there is no difference in glycolytic potential irrespective of sampling immediately after exsanguination or 30 h postmortem. Sampling the day after slaughter is more practical and hygienic, and, therefore, it was chosen for the removal of muscle samples at 22 h postmortem. Muscle lactate, glucose, and glycogen were extracted by homogenizing the samples in 0.6 *M* perchloric acid. After centrifuging the suspension for 10 min at 1,500 × *g*, the supernatant was neutralized with 5 M KOH. The formed potassium perchlorate was removed by centrifugation for 10 min at 1,500 × *g* . Glucose and lactate concentrations were measured using commercially-available kits (NR115 and NR826B for glucose and lactate, respectively; Sigma-Aldrich Chemie BV, Zwijndrecht, The Netherlands). Assays were adjusted for measurement in microtiter plates. Glycogen was enzymatically hydrolyzed to glucose with amyloglucosidase (A7420; Sigma-Aldrich Chemie BV, Zwijndrecht, The Netherlands) according to procedures outlined by Passoneau and Lowry (1993). The glycolytic potential was calculated as the sum of 2 × ([glycogen] + [glucose-6-phosphate] + [glucose]) + [lactate] (Monin and Sellier, 1985).

Measurements

At exsanguination, pH and temperature were measured in blood with a portable pH meter (Portamess 911 pH; Knick Elektronische Messgeräte, Berlin, Germany) equipped with a probe-type glass electrode (LoT406; Mettler Toledo, Greifensee, Switzerland) and a portable thermometer (Hand Held Digital Thermometer; Stekon, Hoofddorp, The Netherlands). At 30 min postmortem, pH and temperature were measured in the longissimus muscle (LM) at the level of the third lumbar vertebra, whereas ultimate pH was measured at the same site at 22 h postmortem. The day after slaughter (22 h postmortem), final meat quality measurements were collected in the same region of the LM, except for pork color: Objective color (L*, a*, and b*) was measured on a freshly cut surface at the height of the last lumbar vertebra after a 10-min blooming period with a Minolta Portable Chroma Meter (Model CR 210; Minolta, Osaka, Japan) equipped with a 50-mm aperture and using illuminant D65. Internal light scattering was measured using the Fiber Optic Probe (TBL Fibres, Leeds, UK), and electrical conductivity was measured using the LF-Star (Ingenieurbüro Matthäus, Nobitz, Germany). Water-holding capacity of the LM was measured by two methods. A filter paper (45-mm diameter) was weighed, gently pressed on the caudal cut surface of the LM for 10 s, and subsequently reweighed to determine the absorbed moisture content. Additionally, a slice of the LM was placed with a cut surface facing down on a metal grid in a closed plastic container. Drip loss was determined as percentage of weight loss after 24 and 48 h of storage.

Statistical analysis

Data were analyzed by the mixed-model procedure (PROC MIXED) of SAS (version 8.02; SAS Inst., Inc., Cary, NC). Least squares means were generated by the LSMEANS statement. Tests of multiple comparisons of LSMEANS were adjusted according to the Tukey-Kramer method to ensure the overall significance level of $P = 0.05$. The model applied for plasma cortisol, lactate, and catecholamines included the fixed effects of stress level and the random effect of slaughter day. Blood hormones and metabolites were analyzed, separately, within plant because CO_2 stunning, as opposed to electrical stunning, caused an inevitable time lag between the onset of the stressor and the moment of blood sampling (3 min from entering stunning equipment until exsanguination). The model applied for temperature and pH measured in blood and the LM, as well as for all pork quality attributes measured in the LM, included the fixed effects of stress level and processing plant, as well as their interaction, and the random effect of slaughter day. After correction for plant effects, equations for prediction of drip loss and pork color (L* value) were developed using PROC STEPWISE with the maximum R^2 option (MAXR). Only equations with significant ($P < 0.05$) variables were used to determine the combination of variables leading to the highest R^2.

Results

Results of analyses of plasma catecholamines, cortisol, and lactate are shown in Table 1. At both plants, high preslaughter stress led to higher ($P < 0.001$) plasma cortisol and lactate concentrations, whereas norepinephrine was increased ($P < 0.01$) in highly pigs when processed at plant A but not ($P = 0.787$) at plant B. Plasma epinephrine levels were unchanged ($P > 0.05$) by stressor treatment, regardless of where pigs were slaughtered. Interestingly, cortisol and lactate concentrations were of the same order at both plants, but epinephrine concentrations were two to three times higher, and norepinephrine levels six to nine times higher, at Plant B than at Plant A.

Table 1. The effect of preslaughter stress level on plasma concentrations of epinephrine, norepinephrine, cortisol, and lactate at two processing plants

	Plant A				Plant B			
	Stress				Stress			
	Minimal	High	Pooled SE	*P*-value	Minimal	High	Pooled SE	*P*-value
No. of animals	47	45	--	--	49	48	--	--
Epinephrine, ng/mL	69.0	85.2	15.2	0.120	216.4	231.4	34.9	0.363
Norepinephrine, ng/mL	37.7	55.8	8.0	0.004	346.1	338.8	105.8	0.787
Cortisol, ng/mL	67.9	80.8	4.1	0.017	49.5	72.1	7.0	0.001
Lactate, mmol/L	15.6	27.7	1.0	0.001	11.9	21.3	2.0	0.001

Blood and muscle temperature, pH values, and LM glycolytic potential are presented in Table 2. There were stress × plant interactions for blood and muscle temperature ($P = 0.057$ and 0.072, respectively), as well as for blood pH ($P < 0.001$). At Plant A, high preslaughter stress led to a higher blood temperature at exsanguination; however, blood temperature was similar for both stress levels at plant B. Moreover, temperatures were lower ($P = 0.084$) at Plant B than Plant A. A similar stress × plant interaction ($P < 0.001$) was found for blood pH, with pigs subjected to the high preslaughter stress having lower blood pH values at plant A, whereas stressor treatments did not alter blood pH at plant B. Muscle temperatures, on the other hand, were increased ($P < 0.001$) at 30 min postmortem by high preslaughter stress at both plants, but differences between minimal and high preslaughter stress were greater at Plant A than Plant B. At both plants, muscle pH values were lower ($P < 0.001$) at 30 min postmortem in pigs subjected to high preslaughter stress; however, ultimate (22 h) pH values were higher ($P < 0.05$) for pigs of the high stressor treatment. Even though glycolytic potential

values were different ($P < 0.05$) between stress levels, minimally-stressed pigs had higher ($P < 0.05$) glycolytic potentials than highly stressed pigs only at Plant A.

Pork quality attributes are displayed in Table 3. Except for redness (a*; $P < 0.01$), no ($P > 0.05$) interactions were observed between plant and stressor level for any pork quality trait. High, compared with minimal, preslaughter stress increased ($P < 0.05$) internal reflectance, L* values, electrical conductivity, filter paper moisture, and both 24- and 48-h drip loss percents. Yellowness (b*) did not ($P > 0.05$) differ between stress levels or plants; however, a* values were lower at Plant B than A ($P < 0.01$) and, additionally, were increased ($P < 0.01$) by high preslaughter stress only at Plant B. Water-holding capacity, as measured by filter paper moisture, was more favorable ($P < 0.01$) at plant B than A, but both 24- and 48-h drip loss percents and electrical conductivity were similar ($P > 0.05$), regardless of where pigs were slaughtered.

Table 2. The effect of plant (P) and preslaughter stress level (S) on temperature and pH of blood (at exsanguination) and in the LM, as well as glycolytic potential of the LM

	Plant					*P*-value		
	A		B					
Stress:	Minimal	High	Minimal	High	Pooled SE	S	P	S × P
No. of carcasses[a]	47	45	49	48	--	--	--	--
Temperature, °C								
Blood	39.6^{x}	40.0^{y}	39.1^{x}	39.2^{x}	0.233	0.004	0.084	0.057
30-min LM	39.7^{xy}	40.9^{z}	39.2^{x}	40.0^{yz}	0.278	0.001	0.121	0.072
pH								
Blood	7.17^{y}	7.03^{x}	6.96^{x}	6.97^{x}	0.072	0.001	0.231	0.001
30-min LM	6.56	6.28	6.62	6.39	0.076	0.001	0.413	0.562
22-h LM (ultimate)	5.55	5.62	5.60	5.64	0.049	0.012	0.555	0.441
Glycolytic potential, µmol/g	137.2	131.0	130.6	128.2	11.49	0.010	0.184	0.261

[a]For 30-min LM pH, n = 38, 43, 47, and 46/treatment, respectively.

[x,y,z]Within a row, least squares means lacking a common superscript letter differ ($P < 0.05$).

Regression analysis revealed that both lactate and glycolytic potential explained a considerable portion of the variation in drip loss and L*. The equation accounting for the greatest amount of variation in drip loss ($R^2 = 0.58$) included blood lactate and temperature, glycolytic potential, and LM pH and temperature measured 30 min postmortem (Table 4). For L* value, glycolytic potential, alone explained about 32% of

the variation, whereas including the variables of blood lactate and LM pH measured 30 min postmortem in the model with glycolytic potential accounted for 50% of the variation in L* values.

Table 3. The effect of plant (P) and preslaughter stress level (S) on pork quality attributes of the LM

	Plant						
	A		B			*P*-value	
Stress:	Minimal	High	Minimal	High	Pooled SE	S	P
No. of carcasses[a]	47	45	47	48	--	--	--
FOP[b]	34	40	39	43	4.7	0.005	0.592
L*[c]	50.9	52.4	51.2	51.6	0.96	0.040	0.834
a*[c,d]	17.9[z]	17.7[z]	15.4[x]	16.1[y]	0.33	0.100	0.006
b*[c]	6.4	6.3	5.8	5.9	0.22	0.867	0.143
EC[e], mS	5.9	9.2	6.6	9.0	0.52	0.001	0.685
FPM[f], mg	89	130	59	100	6.6	0.001	0.006
Drip loss[g], %							
24 h	1.36	1.90	1.08	1.91	0.292	0.001	0.738
48 h	2.27	2.97	1.90	2.76	1.261	0.001	0.598

[a]For meat color measurements (L*, a*, b*), n = 36, 33 47, and 48/treatment, respectively.
[b]FOP = fiber optic-measured light scattering (higher value is indicative of greater light scattering and paler color)
[c]L* = a measure of darkness-lightness (higher value indicates a lighter color); a* = a measure of redness (higher value indicates a redder color); and b* = a measure of yellowness (higher value indicates a more yellow color).
[d]Stress × plant interaction ($P = 0.007$).
[e]EC = electrical conductivity (a higher value indicates a lower water-holding capacity).
[f]FPM = filter-paper measured moisture content.
[g]Drip loss percentages were calculated after storage at 4°C for either 24 or 48 h.
[x,y,z]Within a row, least squares means lacking a common superscript letter differ ($P < 0.05$).

Discussion

Effect of stress on pork quality and blood and muscle pH and temperature

Results of this experiment confirm the detrimental effect of stress on meat quality (van der Wal et al., 1999; Channon et al., 2000). The tenet that high temperatures, in combination with low pH values, in postmortem muscle are responsible for meat quality defects is widely accepted (Wismer-Pedersen and Briskey, 1961; Briskey, 1964;

Offer 1991). In agreement, the inferior pork quality observed for the high preslaughter stress pigs was associated with an increased temperature and a lower pH at 30 min postmortem. The differences in temperature and pH between high and minimal preslaughter stress could already be measured in blood at exsanguination at plant A after electrical stunning, but not at Plant B after CO_2 stunning. It is unclear why the high stressor treatment increased blood temperature and decreased blood pH at Plant A, but not at Plant B, because there is sufficient evidence that numerous different stressors elevate body temperature (Geers et al., 1994; Veum et al., 1979; Judge et al., 1973) and decrease blood pH (Kallweit, 1982; Veum et al., 1979; Judge et al., 1973) . However, the lower blood pH level for the minimally stressed pigs at Plant B, compared with Plant A, may be explained by the different methods of stunning. Forslid and Augustinsson (1988) and Overstreet et al. (1975) showed that inhalation of CO_2 lowers blood pH by the formation of HCO_3^-, which leads to a systematically lower blood pH after CO_2 stunning compared with electrical stunning.

Effect of stress on cortisol, lactate and catecholamines

High preslaughter stress, commencing only 5 min before slaughter resulted in higher blood cortisol levels at both plants, which is consistent with other experiments (Becker et al., 1985; Jensen-Waern and Nyberg, 1993). Both physical exercise (Jensen-Waern and Nyberg, 1993; Steinhardt and Löwe, 1985) and psychological stress (Neubert et al., 1996; Fernandez et al., 1994) may increase lactate concentrations. Accordingly, results of the present experiment are consistent with other studies reporting higher blood lactate values as a result of preslaughter stress (Warris et al., 1994; Hartung et al., 1997; Brown et al., 1998). At both plants, a similar stress response could be observed for cortisol and lactate, although effects of the different stunning methods cannot be completely ruled out. Norepinephrine, on the other hand, was increased in highly stressed pigs slaughtered at Plant A, but not at Plant B. Moreover, both norepinephrine and epinephrine levels were much higher at Plant B, suggesting a higher stress level in the immediate preslaughter period. This was, however, not confirmed by blood lactate and cortisol levels or by pork quality traits, which were more favorable at Plant B. Results of Althen et al. (1977) and Troeger and Woltersdorf (1991) also suggest that the extremely high catecholamine levels after stunning, particularly after CO_2 stunning, are independent of the stressful events that pigs experience before slaughter, but are the consequence of the act of stunning.

Effect of stress on glycolytic potential

Stress, as applied in the present experiment, was related to physical activity and energy consuming. As a consequence, glycolytic potential (a good approximation of muscle glycogen at slaughter) was reduced by high preslaughter stress. This is in agreement with D'Souza et al. (1998), who observed a decrease in muscle glycogen after application of electric shocks before slaughter.

Table 4. Single and multiple variable prediction equations for drip loss percents after 24 h of storage and lightness (L*) values

	Drip loss, %				Lightness (L* value)		
Equation:	1	2	3	4	1	2	3
R2	0.32	0.52	0.56	0.58	0.32	0.48	0.50
Intercept[a]	-0.032	-0.048	-0.063	-0.071	-0.201	-0.279	-0.311
Variables[b]							
Blood lactate	0.076	0.076	0.056	0.044	--	0.156	0.120
Blood temperature	--	--	--	-0.213	--	--	--
Glycolytic potential	--	0.041	0.037	0.039	0.156	0.164	0.148
30-min LM pH	--	--	-0.914	-0.976	--	--	-1.969
30-min LM temperature	--	--	--	0.244	--	--	--

[a]The intercept was not different from zero ($P > 0.05$) in any equation.
[b]Variables were corrected for plant effects, and all variables included in each model were significant ($P < 0.05$).

Relationship between stress indicators and meat quality

Blood pH, epinephrine, and norepinephrine were not considered in the regression analysis because they appeared to be influenced more by the stunning system than by the stress experienced before stunning. Although clearly increased by preslaughter stress, cortisol did not contribute to the explanation of variation in either drip loss or pork color. This may be due to the fact that cortisol is raised by long-term stress (e.g., transport and fighting) leading to DFD meat (Warris and Brown, 1985; Fernandez and Tornberg, 1991), as well as by short-term stress, which is more related to PSE meat. Results of the present experiment are in agreement with studies of Shaw et al. (1995) and Warriss et al. (1998), who could not establish a relationship between cortisol levels and the occurrence of PSE meat.

Variation in glycolytic potential accounted for a considerable portion of the variation in pork quality. Rosenvold et al. (2001b), Schäfer et al. (2002), and Henckel et al. (2002) indicated that the level of glycogen was related to meat quality only if extremely low and/or extremely high levels were found in muscle. Low glycolytic potentials may be caused by energy depletion in response to exhaustive events preslaughter (Fernandez and Tornberg, 1991), whereas extremely high levels are related to the rendement napole (*RN*) gene (Monin and Sellier, 1985; van Laack and Kauffman, 1999). In the present experiment, however, none of the experimental pigs exceeded the value of 180 to 200 μmol/g wet tissue, used to identify *RN* carriers (Fernandez et al., 1992; van

Laack and Kauffman, 1999; Miller et al., 2000). Moreover, ultimate LM pH was well below the frequently used limit of 6.0, indicating that muscle energy had not been depleted before slaughter. Although within a normal range of glycolytic potential values, muscle energy had a clear impact on drip loss and on meat color, with low muscle energy levels being associated with lower drip losses and darker pork color. Figure 1 presents the relationship between glycolytic potential and drip loss for the high and minimal stress groups. The relationship was clearly distinct for the two stress levels but, nevertheless, followed a similar linear pattern over the range of glycolytic potentials. The differences in results between the aforementioned (Rosenvold et al., 2001b; Schäfer et al., 2002; Henckel et al., 2002) and present studies may be attributed to different experimental conditions. In the present study there was variation in genotype, feed withdrawal, and lairage times between groups of pigs, which possibly influenced muscle energy level at slaughter differently than in experiments with greater control over experimental treatments/conditions designed to reduce animal-to-animal and/or treatment variation (Rosenvold et al. 2001b; Schäfer et al., 2002; Henckel et al., 2002).

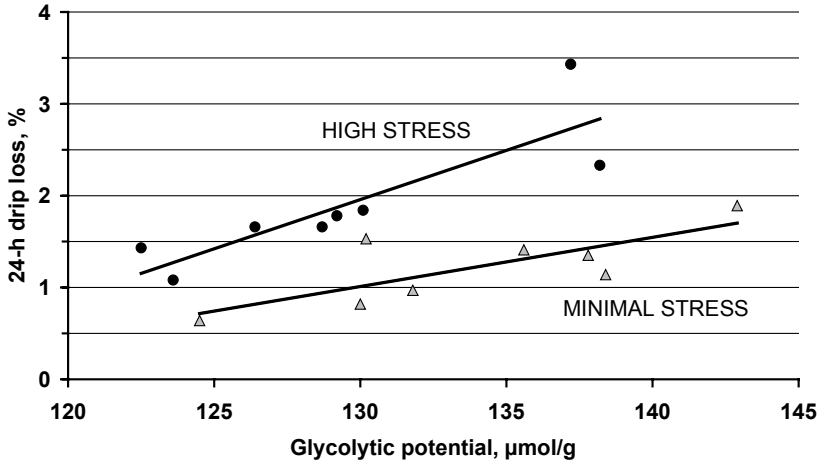

Figure 1. Relationship between glycolytic potential and 24-h drip loss. Each point represents the mean of 12 pigs of the same preslaughter stress level (black circles = high stress and gray triangles = minimal stress) processed on the same day.

The introduction of 30-min LM pH into both the drip loss and L* prediction equations, as well as the introduction of 30-min LM temperature into the drip loss prediction equation, increased R^2 only slightly and changed the regression coefficient of blood lactate. Indeed, blood lactate and 30-min LM pH, and blood lactate and 30-min LM temperature were highly correlated (r = -0.60 and 0.65, respectively; results not shown),

indicating that they explain, to some extent, the variation in drip loss and pork color by a similar mechanism (an increase in both antemortem and postmortem metabolism). A major advantage of monitoring stress by blood lactate levels, as opposed to muscle pH and temperature measurements, could be the time of sampling. Blood lactate would not be influenced by post-stunning handling, such as shackling, scalding and singeing; however, post-stunning handling of carcasses can affect muscle pH and temperature commonly measured at 30 or 45 min postmortem (Aalhus et al., 1991; van der Wal et al., 1995a; Maribo et al., 1998).

In contrast to the regression analysis for drip loss, muscle energy accounted for a greater portion of the variation in LM L* values than did either blood lactate or 30-min LM pH. The effect of glycolytic potential on both pork color and drip loss was probably, at least partly, mediated by its relation with ultimate pH ($r = -0.71$; results not shown). Consequently, the effect of glycolytic potential on pork color can be explained by altered absorption characteristics of myoglobin and reduced light scatter of the meat surface associated with increasing ultimate pH values (Lawrie, 1998). In agreement with the results from the present study, Rosenvold and Andersen (2003) failed to find a relationship between early postmortem pH decline and pork color.

The increase in drip loss with increasing glycolytic potential was more pronounced in the high stress group (Figure 1). Obviously, preslaughter stress had a greater effect on meat quality when muscle energy was high, which is visualized in Figure 2 for drip losses. Individual pigs were, independently from preslaughter stress level and processing plant, classified according to their blood lactate level and glycolytic potential. High glycolytic potentials alone caused only a moderate increase in drip losses; however, when in the presence of high muscle energy, the effect of preslaughter stress on drip loss was exacerbated. The reason why high muscle energy levels appear to be a risk factor when coinciding with high preslaughter stress is probably associated with its relation to ultimate pH. According to Offer and Knight (1988), water is held by the internal structure of myofibrils. Preslaughter stress causes a more rapid pH decline and higher muscle temperatures, which results in myofibrillar protein denaturation (Offer and Knight, 1988; Offer, 1991). As a consequence, water is less firmly held by that internal structure (Offer and Knight, 1988). Then, this loosely held water may be actually lost as muscle pH approaches the isoelectric point of muscle proteins (pH 5.0; Hamm, 1960). Thus, in the present study, low muscle glycolytic potentials may have led to a higher ultimate pH, partly preventing water from being expelled from the meat. The proposed mechanism by which muscle energy influenced drip losses and pork color in the present study is different but not in contradiction to Rosenvold et al. (2003), who suggested that pork quality was affected by an alteration of glycometabolism rather than changes in ultimate pH.

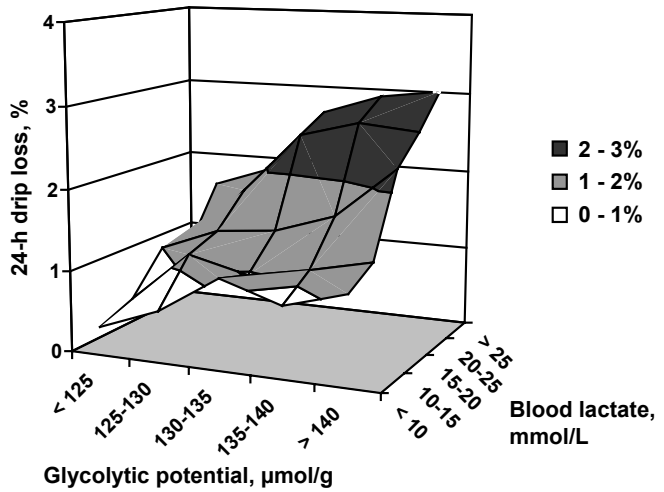

Figure 2. Relationship of the combination of blood lactate and glycolytic potential with drip loss after 24 h of storage. Individual data for lactate and glycolytic potential were grouped into classes. Drip loss values represent the average of all pigs with the respective lactate and glycolytic potential class (3 to 14 pigs/class).

The importance of both the rate and the extent of postmortem glycolysis in drip formation and pork color has been long recognized (e.g. Wismer-Pedersen and Briskey, 1961; Briskey, 1964; Offer and Knight, 1988). However, the present results provide additional evidence that this is true in pig populations free of both the halothane and the *RN* genes. Moreover, in contrast to other studies (Rosenvold et al., 2001b; Schäfer et al., 2002; Henckel et al., 2002), the importance of muscle energy level at slaughter in relation to drip losses and pork color is emphasized.

Implications

Stress immediately before slaughter has severe, negative consequences on pork quality attributes, such as drip loss and pork color, both after electrical and CO_2 stunning. The negative effects of stress may be aggravated by high muscle energy levels present at slaughter. Strategies are needed to monitor and reduce preslaughter stress, on the one hand, and to control muscle energy, on the other. Blood lactate seems to be a promising indicator of both the physical and psychological stress associated with the handling of pigs immediately before slaughter.

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Negative effects of stress immediately before slaughter on pork quality are aggravated by suboptimal transport and lairage conditions

5

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Abstract

The objectives of the present experiment were to: 1) study the effects of transport conditions and lairage duration on stress level, muscle glycolytic potential, and pork quality, and 2) investigate whether the negative impacts of high stress immediately preslaughter may be affected by preceding handling factors (transport and lairage). In a 2 × 2 × 2 factorial design, halothane-free pigs (n = 384) were assigned to either short (50 min) and smooth or long (3 h) and rough transport, long (3 h) or short (< 45 min) lairage, and minimal or high preslaughter stress. Pigs were processed in 8 groups (48 pigs/group) on various days at a commercial plant. Blood samples were taken at exsanguination to measure plasma cortisol and lactate. Muscle pH and temperature were measured at 30 and 40 min, respectively, and both at 3 h, postmortem. A longissimus muscle (LM) sample was taken at 135 min postmortem to estimate glycogen content and rate of glycolysis. Pork quality attributes were assessed 23 h postmortem. Long transport, but not lairage ($P > 0.30$), tended to increase ($P = 0.06$) muscle glycolytic potential. Long transport tended to increase ($P = 0.08$) electrical conductivity, and reduced a* ($P < 0.01$) and b* ($P < 0.02$) values. Reducing lairage from 3 h to < 45 min decreased ($P < 0.05$) the L* value but did not ($P > 0.10$) affect other pork quality traits. High stress decreased ($P < 0.001$) muscle glycolytic potential, and increased ($P < 0.001$) plasma lactate, cortisol, muscle temperature, rate of pH decline, and ultimate pH. Except for decreased ($P < 0.001$) b* values, pork color was not ($P > 0.40$) affected by high stress, but water-holding properties (measured by electrical conductivity, filter paper moisture, and drip loss) were impaired ($P < 0.001$) by high stress. Fibre optic-measured light scattering and Warner-Bratzler shear force were not ($P > 0.12$) affected by any treatment. Comparisons with the "optimal" handling (short transport, long lairage, and minimal stress) revealed that, with regards to water-holding properties, the negative effects of high stress were aggravated by suboptimal transport and lairage conditions. High stress alone increased electrical conductivity by 56%, whereas high stress, in combination with short lairage, led to an 88%-increase. However, high stress contributed most and was the major factor responsible for reductions in pork quality.

Introduction

The rate and extent of postmortem acidification in pork muscles largely determine ultimate meat quality (Wismer-Pedersen and Briskey, 1961; Briskey, 1964; Offer and Knight, 1988). Van der Wal et al. (1999), Rosenvold et al. (2001), and Hambrecht et al. (2004b) found that preslaughter stress level and glycolytic potential (a measure for the in vivo muscle glycogen content present before slaughter) are closely related to these metabolic processes. In fact, both preslaughter stress and glycolytic potential explain a large portion of the variation in drip losses and pork color. Research has shown that genetic variation independent from the *RN* gene (Ciobanu et al., 2001; Oksbjerg et al., 2001), fasting (Eikelenboom et al., 1991; Wittman et al., 1994), transport (Becker et al., 1985; Geers et al., 1994; Leheska et al., 2003), and lairage (Honkavaara, 1989; Nanni Costa et al., 2002; Pérez et al., 2002b) may influence muscle glycolytic potential and/or stress level, and, ultimately, pork quality. Hambrecht et al. (2004b) concluded that differences in any one, or several, of these factors may markedly impact muscle glycolytic potential, but which factor accounts for the greatest amount of variation in postmortem glycolysis is still largely unknown. Therefore, the present experiment was designed to study the effects of transport conditions and lairage duration on stress level, muscle glycolytic potential, and ultimate pork quality under controlled conditions. The objective was to test whether the negative effects of high stress immediately before slaughter were affected by preceding handling factors, such as transport and/or lairage.

Materials and methods

The experimental protocol was approved by the Animal Care and Ethics Committee of the University of Nijmegen, The Netherlands.

Animals and experimental design

All pigs were commercial, Yorkshire × (Large White × Landrace), and halothane-free endproducts of the *Hypor* pig breeding company (Regina, Canada). In a completely randomized design, barrows and gilts (n = 384) were assigned to one of eight treatments arranged in a 2 × 2 × 2 factorial design, with two types of transport (short and smooth or long and rough), two lairage durations (long or short), and two stress levels immediately before slaughter (minimal or high). Eight groups of 48 pigs, all originating from the same commercial farm, were processed during eight weeks on various days. Long and short lairage alternated between one week and the next, whereas transport types and preslaughter stress levels were varied within the same slaughter day. The experiment took place from October to December. On six processing days, outdoor temperatures ranged between 5 and 12°C during the relevant periods of transport and lairage. Only on two consecutive slaughter occasions, outdoor temperatures fell below the freezing point (-4 to 0°C).

Preslaughter and slaughter

All pigs were fed the same commercial diet in a liquid feeding system, and received their last meal 16 h before slaughter. Within each processing day, four groups of pigs, each group consisting of 12 pigs of three different pens (4 pigs/pen out of a total of 12 pigs/pen), were randomly assigned in their home pen to either short and smooth or long and rough transport and either minimal or high preslaughter stress. Loading density was 0.45 m^2/100 kg BW for all pigs. The first two treatments (minimal and high preslaughter stress after long transport) were loaded and driven around for 2 h on minor roads with frequent roundabouts and speed bumps, resulting in the truck changing speed and periodic stopping/starting. Meanwhile, pigs of the other two treatments (minimal and high preslaughter stress after short transport) were loaded on a second, larger, but otherwise similar, truck (double-deck truck with all pigs loaded onto the upper deck). After returning to the farm, the two groups of the long transport treatment were unloaded and reloaded onto the second truck, and transported together with the two groups of pigs of the short transport treatment to the processing plant. This transport was mostly smooth (paved, four-lane highways), and took approximately 50 min. After arrival, pigs were kept within their treatments in seperate holding pens at a stocking density of 0.75 m^2/100 kg BW. Pigs in the long lairage treatment received a 3-h rest before slaughter, whereas pigs that were subjected to the short lairage treatment were only rested for 30 to 45 min. Experimental pigs were the first to be slaughtered at 0630, which meant that for the most part lights were dimmed in lairage and there was little noise. All pigs were showered for approximately 10 min directly before they were led to the stunning area. Experimental stressor treatments were the same as described by Hambrecht et al. (2004b). Pigs in the minimal stress group were guided to the stunning area without the use of electric goads and were handled as calmly as possible, whereas pigs in the high stressor treatments were forced, by yells and electric goads, to move four times back and forth in the corridor leading to the stunning area. Pigs were electrically stunned in a fully automated, head-to-heart stunning system (MIDAS, Stork, The Netherlands), and care was taken that all pigs were shackled using the right hind leg.

Sampling procedures and chemical analyses

At exsanguination, a 9-mL blood sample was collected in a heparinized tube (Monovette LH; Sarstedt, Nümbrecht, Germany) from each pig for cortisol and lactate determinations. Blood samples were immediately put on ice and transported back to the laboratory, where samples were centrifuged for 10 min at 1,300 × *g*, and plasma (1.0 mL) was transferred to Eppendorf tubes and stored at -20°C until analysis. Plasma cortisol concentrations were determined according to Erkens et al. (1998) using a solid-phase RIA kit (Coat-a-Count Cortisol TKCO; Diagnostic Products Corporation, Apeldoorn, The Netherlands). Blood lactate levels were determined spectro-photometrically with lactate dehydrogenase and NAD (Bergmeyer, 1974).

At 135 min postmortem, when carcasses had passed from the rapid chilling tunnel into the cooler, muscle samples were taken from the left longissimus muscle (LM) at the last rib for determination of the glycolytic potential. Samples were immediately frozen in liquid nitrogen and stored at -80°C until further analysis. Muscle lactate and glycogen were extracted by homogenizing the samples in 0.85 *M* perchloric acid. After centrifuging the suspension for 10 min at 1,500 × *g*, the supernatant was neutralized with 10 *M* KOH. Lactate in the supernatant was determined spectrophotometrically with lactate dehydrogenase and NAD (Bergmeyer, 1974). Glycogen in the supernatant was enzymatically hydrolysed to glucose by incubation with amyloglucosidase (Sigma A 7420; Sigma-Aldrich Chemie BV, Zwijndrecht, The Netherlands) in acetate buffer (pH 4.8) at 55°C for 2 h. After incubation, the supernatant was neutralized with 10 *M* KOH, and glucose was determined spectrophotometrically by the hexokinase method (Bergmeyer, 1974). The concentrations of glucose-6-phosphate and glucose were not separately determined but are included in the glycogen determination. The glycolytic potential was calculated as the sum of 2 × [glycogen] + [lactate] (Monin and Sellier, 1985).

Measurements

The left carcass side was used for all measurements. Muscle pH and temperature were measured in the LM at the level of the third lumbar vertebra. At 30 min, 3 h, and 24 h postmortem pH was measured using a portable pH meter (Portamess 911 pH; Knick Elektronische Messgeräte, Berlin, Germany) equipped with a probe-type glass electrode (LoT406; Mettler Toledo, Switzerland). Data loggers (Diligence EV N2002; Comark Instruments, Stevenage, UK) equipped with a food penetration probe (PX22L/C; Comark Instruments) to record temperature were inserted in the LM at 40 min postmortem, and temperature was recorded at 5-min intervals until 23 h postmortem; however, only data corresponding with the pH measurement times are reported.

At 23 h postmortem, loins were fabricated for final meat quality measurements. Objective color (L*, a*, and b*) was measured on a freshly cut surface at the height of the last lumbar vertebra after a 10-min blooming period with a Minolta Portable Chroma Meter (Model CR 210; Minolta, Osaka, Japan) equipped with a 50-mm aperture and using illuminant D65. Internal light scattering was measured using the Fiber Optic Probe (TBL Fibres, Leeds, UK), and electrical conductivity was measured using the LF-Star (Ingenieurbüro Matthäus, Nobitz, Germany). Water-holding capacity of the LM was measured by two methods. A filter paper (45-mm diameter) was weighed, gently pressed on the caudal cut surface of the LM for 10 s, and subsequently reweighed to determine the absorbed moisture content. Additionally, a slice of the LM was placed with the cut surface facing down on a metal grid that was placed in a closed plastic container. Drip loss was determined as percentage of weight loss after 24 h of storage. A 2.5-cm LM slice was removed adjacent to the slice used for the drip loss measurement for determination of Warner Bratzler shear force. Samples were vacuum

packed, heated in water at 75°C until an internal temperature of 70°C was reached. Samples were cooled in water until they had reached a temperature of approximately 20°C. Six 1-cm-diameter cores were removed parallel to the length of the muscle fiber and sheared once across the center using a TA-XT2 Texture analyser (Stable Micro Systems, Etten-Leur, The Netherlands). The mean shear force value for each LM slice was used for statistical analysis.

Statistical analysis

Data were analyzed by the mixed-model procedure (PROC MIXED) of SAS (version 8.02; SAS Inst., Inc., Cary, NC). Least squares means were generated by the LSMEANS statement. Tests of multiple comparisons of LSMEANS were adjusted according to the TUKEY-KRAMER method to ensure the overall significance level of $P = 0.05$. The model applied included the fixed effects of transport type, lairage duration, and stress level, as well as their 2-way interactions, and the random effect of slaughter day nested within lairage duration.

Results

Average hot carcass weight was 88.6 kg and lean percentage was 56.7, measured by the Hennessey Grading probe. None of the experimental treatments affected carcass traits ($P > 0.05$; results not shown).

Plasma concentrations of cortisol and lactate, as well as muscle metabolites, are displayed in Table 1. Both plasma cortisol and lactate were increased ($P < 0.001$) by the high stressor treatment, but were not affected by type of transportation ($P > 0.45$) or lairage duration ($P > 0.20$). Even though there were transport × stress ($P = 0.013$) and lairage × stress ($P < 0.001$) interactive effects on plasma lactate content, lactate levels were always higher in plasma of pigs subjected to the high preslaughter stressor level compared to minimally handled pigs (results not shown). Furthermore, a transport × lairage interaction ($P = 0.012$) was detected for plasma lactate concentration, but plasma lactate levels were similar ($P > 0.05$) among the transport and lairage treatment combinations (results not shown).

Even though LM glycogen content was not affected ($P = 0.62$) by the long and rough transport, muscle lactate concentration was increased ($P < 0.01$) by longer, rougher transportation, resulting in greater ($P = 0.06$) glycolytic potentials (Table 1). Lairage had no ($P > 0.15$) effect on muscle metabolites. The high stressor treatment, on the other hand, clearly decreased ($P < 0.001$) muscle glycogen, and increased ($P < 0.001$) LM lactate concentrations, which was reflected by a decrease ($P < 0.001$) in glycolytic potential.

Results of the temperature and pH measurements in the LM are shown in Table 2. Long and rough transport tended to lower ($P = 0.08$) pH at 30 min postmortem.

Table 1. Least squares means for plasma concentrations of cortisol and lactate at exsanguination and LM metabolites at 135 min postmortem as affected by transport (T), lairage (L) or preslaughter stressor level (S)

	Transport		Lairage		Stress			*P*-value		
Item	Short	Long	Long	Short	Minimal	High	Pooled SE	T	L	S
No. of animals	174	184	179	179	176	181	-	-	-	-
Plasma										
Cortisol, ng/mL	67.5	65.5	61.4	71.7	55.1	77.9	4.28	0.434	0.201	0.001
Lactate, mmol/L	23.8	24.1	24.4	23.5	17.1	30.9	0.61	0.617	0.401	0.001
Longissimus muscle										
Glycolytic potential, μmol/g	129.4	132.5	132.2	129.7	134.1	127.8	1.45	0.060	0.304	0.001
Glycogen, μmol/g[a]	29.6	29.1	30.1	28.6	38.0	20.7	0.77	0.621	0.155	0.001
Lactate, μmol/g	70.2	74.3	72.0	72.6	58.1	86.4	1.08	0.008	0.736	0.001

[a]Includes glucose and glucose-6-phosphate concentrations.

There were no ($P > 0.20$) effects of lairage duration on LM pH or temperature. Transport and lairage treatments did not affect ($P > 0.48$) ultimate (24-h) pH values of the LM. However, the high stressor treatment decreased ($P < 0.001$) LM pH at both 30 min and 3 h postmortem, and increased ($P < 0.001$) ultimate (24-h) pH. Muscle temperature was increased ($P < 0.001$) by high preslaughter stress at both 40 min and 3 h postmortem. At 3 h postmortem, the stress-related increase was 1.9 °C after long lairage, approximately twice that of pigs subjected to short lairage (lairage × stress; $P = 0.042$; results not shown).

Pork quality attributes are presented in Table 3. Long and rough transport decreased the redness (a*; $P < 0.01$) and yellowness (b*; $P < 0.02$), and tended to increase ($P = 0.08$) electrical conductivity, of the LM. Lairage was the only treatment factor that affected the lightness (L*) of the meat, with the short lairage treatment resulting in a darker (lower L* values; $P < 0.03$) LM than the long lairage treatment. Even though there was a transport × lairage interaction ($P < 0.011$) for filter paper-measured moisture, differences were small and insignificant ($P > 0.05$; results not shown). The high stressor treatment increased ($P < 0.001$) electrical conductivity, filter paper-measured moisture, and drip losses. However, preslaughter stress did not ($P > 0.41$) influence L* or a* values but decreased ($P < 0.001$) b* values. Fibre optic-measured light scattering ($P > 0.34$) and Warner-Bratzler shear force ($P > 0.12$) were similar among the treatment combinations.

Discussion

Independent from the transport and lairage treatments, variation in plasma lactate (an indicator of stress level) and muscle glycolytic potential (an indicator of muscle glycogen before slaughter) together explained 49 and 28% of the variation in drip losses and L* values, respectively (results not shown). The results for drip losses were very similar to those of Hambrecht et al. (2004b), who reported that plasma lactate and glycolytic potential accounted for 52 and 48% of the variation in drip loss and L* values, respectively. Overall, both preslaughter stress and muscle glycolytic potential level appear to be of paramount importance for pork quality; thus, the transport and lairage treatments that were employed in the present experiment will be discussed with particular emphasis on their effects on stress response and glycolytic potential.

Effects of transport

Differences between the two transport treatments were small and did not elicit major metabolic changes. The long transport treatment aimed at imposing a higher level of both physical and psychological stress on the pigs than the short transport treatment. This was done not merely by increasing its duration but, rather, by making the journey more unpleasant through frequent braking and accelerating and by selecting minor roads with bends, roundabouts, and speed bumps.

Table 2. Least squares means for postmortem pH and temperature decline in the LM as affected by transport (T), lairage (L) or preslaughter stressor level (S)

	Transport		Lairage		Stress			*P*-value		
Item	Short	Long	Long	Short	Minimal	High	Pooled SE	T	L	S
No. of carcasses	174	184	179	179	176	181	-	-	-	-
pH										
30 min	6.51	6.46	6.49	6.48	6.64	6.33	0.027	0.079	0.740	0.001
3 h	6.10	6.05	6.01	6.14	6.23	5.92	0.050	0.094	0.202	0.001
24 h (ultimate)[a]	5.55	5.55	5.54	5.57	5.52	5.58	0.019	0.883	0.482	0.001
Temperature, °C										
40 min	39.4	39.1	39.0	39.4	38.6	39.8	0.27	0.039	0.427	0.001
3 h	21.2	21.3	21.2	21.3	20.6	21.9	0.34	0.760	0.967	0.001

[a]For 24-h pH, n = 157, 165, 177, 145, 163, and 159 for short and long transport, long and short lairage, and minimal and high stressor level, respectively.

Table 3. Least squares means for LM quality attributes as affected by transport (T), lairage (L) or preslaughter stressor level (S)

	Transport		Lairage		Stress			*P*-value		
Item	Short	Long	Long	Short	Minimal	High	Pooled SE	T	L	S
No. of carcasses	174	184	179	179	176	181	-	-	-	-
FOP[a]	46	45	47	44	45	46	1.8	0.540	0.344	0.630
L*[b]	53.9	53.7	54.3	53.3	53.9	53.7	0.23	0.529	0.029	0.494
a*[b]	19.4	19.1	19.4	19.1	19.2	19.3	0.11	0.008	0.141	0.411
b*[b]	5.5	5.3	5.5	5.2	5.5	5.2	0.10	0.018	0.100	0.001
EC, mS[c]	8.0	8.5	7.9	8.6	6.3	10.2	0.64	0.083	0.536	0.001
FPM, mg[d]	67	65	67	65	53	80	3.6	0.437	0.727	0.001
24-h drip loss, %	1.96	2.01	1.89	2.08	1.25	2.73	0.12	0.757	0.331	0.001
WBSF, kg[e]	5.0	5.1	5.0	5.2	5.0	5.1	0.07	0.239	0.121	0.614

[a]FOP = fiber optic-measured light scattering (higher value indicates greater light scattering and a paler color).
[b]L* = a measure of darkness to lightness (higher value indicates a lighter color); a* = a measure of redness (higher value indicates a redder color); and b* = a measure of yellowness (higher value indicates a more yellow color).
[c]EC = electrical conductivity (higher value indicates a lower water-holding capacity).
[d]FPM = filter paper-measured moisture content.
[e]WBSF = Warner-Bratzler shear force (higher value indicates tougher meat).

In sheep (Ruiz-de-la-Torre et al., 2001) and pigs (Bradshaw et al., 1996) rough (as opposed to smooth) transport resulted in elevated cortisol concentrations. In the present experiment, however, this was not observed. During longer transports, animals have more time to adapt to transportation conditions after the stressful events like removal from their home pens, co-mingling, and loading onto trucks, and actually arrive in a better condition at the processing plant than after a short transport (Augustini and Fischer, 1982; Pérez et al., 2002a). In the present experiment, the last third of the transport was smooth for all pigs, which might have promoted a general habituation to transport in the long transport group. However, the tendency towards a more rapid postmortem acidification of muscle and an increased electrical conductivity for the long and rough transport treatment in combination with long lairage are clues that the long transport might have indeed imposed a somewhat higher stress level on the pigs. Although cortisol measurements do not support the assumption, this stressor must have been of psychological, rather than physical, nature because glycolytic potential tended to be increased (and not decreased) by long and rough transport. Warriss et al. (1983) and Brown et al. (1999) found no effect of transport on muscle glycogen. During low-intensity exercise, fatty acids are the preferred source of energy (Romijn et al., 1993; Klein et al., 1994). In consequence, fat oxidation may have provided sufficient energy for physical movement occurring during transportation, which could explain the absence of an effect of transport on muscle glycogen stores. Conversely, Leheska et al. (2003) showed that transportation dramatically reduced antemortem glycogen reserves prior to slaughter. Differences between Leheska et al. (2003) and the current study might be related to the low environmental temperature of -10°C and the very low loading density in their study. In humans, the higher metabolic rate due to cold exposure increased carbohydrate oxidation almost sevenfold while fat oxidation rose less than twofold (Vallerand and Jacobs, 1989). In the present study, an explanation for the somewhat higher glycolytic potential after long transport may be that pigs of the short transport treatment, left in their pens after the removal of the pigs for the long transport, became agitated by the unusual disturbance and actually became more active than their counterparts that were on transport. The effect of muscle glycogen on pork quality was suggested to be mediated, at least partly, by its relation with ultimate pH (Leheska et al., 2003; Hambrecht et al., 2004b). In the present study, however, ultimate pH was not affected by the type of transport. Thus, the precise physiological effects of transport on pigs could not be conclusively described by measurements of plasma lactate, cortisol, and (or) muscle glycolytic potential.

Effects of lairage

Independent from the type of preceding transport, there were limited effects of reducing lairage duration from 3 h to between 30 and 45 min, which is regarded as too short of a time to allow pigs to recover from previous transport stress (De Smet et al.,

1996; Milligan et al., 1998; Pérez et al., 2002b). The glycolytic potential was similar for both lairage durations. Generally, results of the present study indicate that lairage durations between 30 min and 3 h neither promoted glycogen depletion nor replenishment. There are few studies available that have measured muscle glycogen levels in response to various lairage durations. Honkavaara (1989), Fernandez et al. (1992), and Stalder et al. (1998) compared short (0 to 2.5 h) with long lairage durations (16 to 24 h), and found no or a decreasing effect of long lairage on muscle glycogen reserves. However, Warriss et al. (1999) demonstrated that the effect of lairage on muscle glycogen was non-linear in broilers, and that prolonging lairage durations could abolish initial increases in muscle glycogen. The effects of lairage duration on pork quality, within the rather short ranges that were studied in the present experiment, were not likely mediated by the effects on glycolytic potential.

Effects of stress

Results of the high stressor treatment were very similar to those previously observed and discussed by Hambrecht et al. (2004a,b). Unlike other studies (van der Wal et al. 1999; Hambrecht et al., 2004b), but in agreement with D'Souza et al. (1998) and Channon et al. (2000), meat color appeared to be unaffected by stressor level. For all other traits, including plasma lactate and cortisol, muscle metabolites, pH, and temperature, as well as most pork quality attributes, the effects of preslaughter stress superceded the effects of any preceding treatment such as transport and lairage.

Effects of stress in relation to preceding handling factors

Single and combined effects of the three treatment factors on some of the most important pork quality attributes are displayed in Figure 1, whereas the effects on physiological variables are shown in Figure 2. Because some of the attributes show a far larger variation than others, changes caused by the variation of one or several treatment factors are expressed relative to the pooled standard deviation of the respective trait. Although the effects of short and smooth transport were not unequivocally positive, this treatment, with the 3 h of lairage and minimal stressor treatment combination, was selected to represent the zero-line as "optimal" preslaughter handling. As shown in Figure 1, the worse transport conditions and (or) short lairage alone had little effect on pork quality. However, suboptimal transport and lairage conditions exacerbated the effects of high preslaughter stress, predominantly regarding traits related to water-holding properties. For example, the high stressor treatment alone increased electrical conductivity values by 1.1 SD (56%), compared with the "optimal" treatment; however, when additionally the lairage duration was shortened, the increase in electrical conductivity was 1.7 SD (88%).

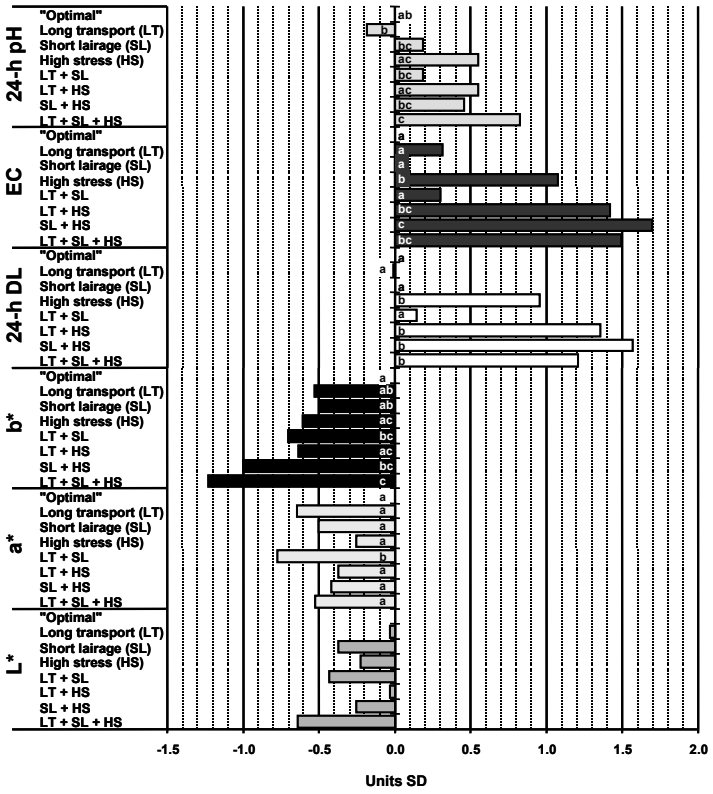

Figure 1. Single and combined effects of preslaughter handling factors on various pork quality attributes. Absolute differences between means were divided by the pooled SD (**SDp**) of all treatment means for the respective attribute. Short (50 min) and smooth transport, long (3 h) lairage, and minimal stress before slaughter were regarded as "optimal" preslaughter treatment and represent the zero-line. Bars indicate deviation from this zero-line by the variation of only one treatment factor (long (3 h) and rough transport, short (< 45 min) lairage, or high preslaughter stress) or the combined variation of several treatment factors. 24-h pH = ultimate pH (SDp = 0.11); EC = electrical conductivity (SDp = 3.04); 24-h DL = drip loss percent after storage for 24 h (SDp = 1.19); L* = a measure of lightness (SDp = 2.68); a* = a measure of redness (SDp = 1.10); b* = a measure of yellowness (SDp = 0.74). Within one attribute, least squares means of the corresponding bars lacking a common letter differ ($P < 0.05$).

These findings were supported by the physiological variables presented in Figure 2. Compared with the effects of high stress alone, both plasma lactate, cortisol, and muscle temperature were increased when concomitantly transport conditions were

worsened and (or) lairage duration decreased, which resulted in an increased rate of postmortem muscle acidification. In agreement, increases in plasma lactate, muscle temperature, and rate of early postmortem glycolysis have been associated with reductions in pork quality (Wismer-Pedersen and Briskey, 1961; Offer 1991; Hambrecht et al., 2004b).

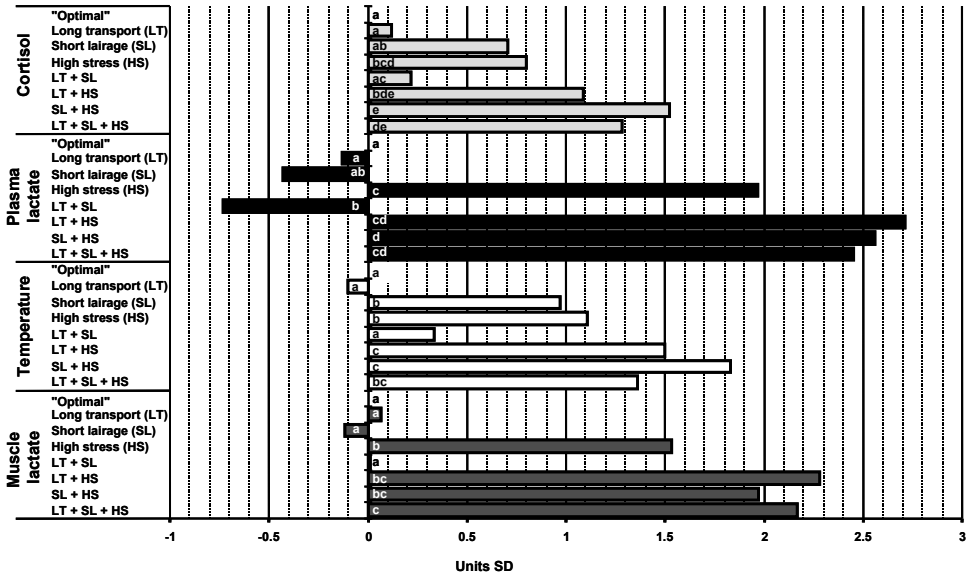

Figure 2. Single and combined effects of preslaughter handling factors on plasma cortisol (pooled SD (**SD_p**) = 24.68) and lactate concentrations (SD_p = 5.02) measured at exsanguination, muscle temperature measured at 40 min postmortem (SD_p = 1.08) as well as muscle lactate measured at 135 min postmortem (SD_p = 14.18). Absolute differences between means were divided by the SD_p of all treatment means for the respective attribute. Short (50 min) and smooth transport, long (3 h) lairage, and minimal stress before slaughter were regarded as "optimal" preslaughter treatment and represent the zero-line. Bars indicate deviation from this zero-line by the variation of only one treatment factor (long (3 h) and rough transport, short (< 45 min) lairage, or high preslaughter stress) or the combined variation of several treatment factors. Within one attribute, least squares means of the corresponding bars lacking a common letter differ ($P < 0.05$).

It is not clear why the long and rough transport or the short lairage treatment, alone, resulted in only limited effects on stress responses and pork quality, but increased stress levels and impaired pork quality when combined with the high preslaughter stressor treatment. Geverink et al. (1998) found that the combination of driving and

mixing of pigs led to a greater increase in cortisol than each individual treatment. Others have noted that animals may react stronger to a stressor when previously sensitized by another stressor (Bruijnzeel et al., 2001; Stam et al., 2000, 2002). These studies investigated the sensitization after a period of 2 to 3 wk; however, it is not known whether sensitization can occur within a matter of hours. Barton Gade (1996) reported an increased degree of aversion of pigs to handling if not sufficiently rested. In the present study, the high stressor treatment was associated with a high degree of coercion; thus, an increased resistance of pigs after short lairage may have actually increased the stress level as experienced by the pigs, thereby aggravating the negative effects of stress on pork quality.

Implications

Transport and lairage conditions, in the range that was investigated in the present study, have little influence on pork quality. However, when coinciding with high stress in the immediate preslaughter period, a long and rough transport, as well as a too short lairage duration, may aggravate negative effects of high preslaughter stress. Largest improvements in pork quality can be achieved by reducing stress in the immediate preslaughter period.

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Preslaughter handling effects on pork quality and glycolytic potential in two muscles differing in fiber type composition

6

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Abstract

The objective of the present experiment was to investigate whether the effects of various levels of physical and psychological stress caused by commercially relevant transport, lairage, and preslaughter stressor treatments depended on the metabolic type of the muscle. For this purpose, the glycolytic potential and pork quality were assessed in the glycolytic longissimus muscle (LM) and the oxidative supraspinatus (SSP) or serratus ventralis (SV) muscles, respectively. In a 2 × 2 × 2 factorial design, 384 pigs were assigned to either short and smooth or long and rough transport (50 min or 3 h), long or short lairage (3 h or < 45 min), and minimal or high preslaughter stress. Muscle samples were taken from the LM at 135 min and from the SSP at 160 min postmortem for determination of the glycolytic potential and rate of glycolysis. Pork quality was assessed in the LM and the SV muscle 23 h postmortem. The effects of transport and lairage conditions were similar in both muscle types. Long vs. short transport increased ($P < 0.01$) the glycolytic potential and muscle lactate. Long transport and short lairage both decreased ($P < 0.01$) redness and yellowness of the meat. In combination with short lairage, long transport decreased ($P < 0.05$) also the lightness. Electrical conductivity was increased ($P < 0.05$) after long transport. For the high preslaughter stressor treatment, on the other hand, several interactions between stress level and muscle type ($P < 0.001$) were observed. High stress decreased ($P < 0.001$) muscle glycogen in both the LM and the SSP muscle but this decrease was larger in the LM. Only in the LM, lactate was increased ($P < 0.001$) by high stress. The decrease in redness ($P < 0.01$) was larger in the SV muscle as well as the increase in ultimate pH ($P < 0.001$). The increase in electrical conductivity ($P < 0.001$) was larger in the LM. The lack of interactions between the transport and lairage treatments and muscle type is attributed to the small differences in physical activity and psychological stress between these treatments. It is concluded that, in glycolytic muscle types such as the LM, the high physical and psychological stress levels associated with stress in the immediate preslaughter period have larger effects on the water-holding capacity of the meat and may promote PSE development. Whereas, oxidative muscle types will tend to high ultimate pH values and DFD pork in response to intense physical activity and (or) high psychological stress levels preslaughter.

Introduction

Various stages during preslaughter handling, such as transport (Pérez et al., 2002a; Leheska et al., 2003), lairage (Milligan et al., 1998; Pérez et al., 2002b) and stress immediately before slaughter (van der Wal et al., 1999; Hambrecht et al., 2004b), were shown to affect pork quality. Most of the effects were attributed to alterations in the rate and extent of pH decline postmortem, which in turn are related to muscle glycolytic potential (an estimate of muscle glycogen in vivo) and a stress-induced increase in glycolysis. Due to its size and accessibility, the longissimus muscle (LM) is the most frequently assessed muscle. However, muscles consist of various fiber types that can be classified on the basis of their contractile and metabolic properties into slow-twitch oxidative and fast-twitch glycolytic types, as well as an intermediate type (Solomon and Dunn, 1988). "Oxidative" compared with "glycolytic" muscle fibers show a lower glycolytic potential (Monin et al., 1987; Fernandez et al., 1994), a lower glycolytic capacity (Vøllestad et al., 1992), a higher sensitivity to epinephrine (Górski, 1978; Fernandez et al., 1995b), and a lower threshold force resulting in an earlier recruitment at low-intensity exercises (Køpke et al., 1984; Lacourt and Tarrant, 1985). It can be expected that muscles, depending on their fiber type composition, react differently to the physical and psychological stressors associated with preslaughter handling. The objective of the present experiment was to investigate whether the effects of various levels of physical and psychological stress caused by commercially relevant transport, lairage, and preslaughter stressor treatments depended on the metabolic type of the muscle. For this purpose, the glycolytic potential and pork quality were assessed in the LM, predominantly consisting of fast-twitch glycolytic fibers, and the supraspinatus or serratus ventralis muscles, respectively, both containing a high portion of slow-twitch oxidative muscle fibers.

Materials and methods

The experimental protocol was approved by the Animal Care and Ethics Committee of the University of Nijmegen, The Netherlands.

Animals and experimental design

All pigs were commercial, Yorkshire × (Large White × Landrace), and halothane-free endproducts of the *Hypor* pig breeding company. In a randomized design, pigs (n = 384, equal numbers of gilts and barrows) were assigned to one of eight treatments in a 2 × 2 × 2 factorial arrangement, with two types of transport (short and smooth or long and rough), two lairage durations (long (considered as optimal) or short (considered as sub-optimal)) and two stress levels immediately before slaughter (minimal or high). Eight groups of 48 pigs, all originating from the same commercial farm, were processed during eight weeks on various days. Long and short lairage alternated between subsequent weeks. Transport types and preslaughter stress levels were varied within

the same slaughter day. The experiment took place from October to December. On six processing days, outdoor temperatures ranged between 5 and 12°C during the relevant periods of transport and lairage. Only on two consecutive slaughter occasions, outdoor temperatures fell below the freezing point (-4 to 0°C).

Preslaughter and slaughter

All pigs were fed the same commercial diet in a liquid feeding system, and received their last meal 16 h before slaughter. Within each processing day, four groups of pigs, each group consisting of 12 pigs of three different pens (4 pigs/pen out of a total of 12 pigs/pen), were randomly assigned in their home pen to either short and smooth or long and rough transport and either minimal or high preslaughter stress. Loading density was 0.45 m^2/100 kg BW for all pigs. The first two treatments (minimal and high preslaughter stress after long transport) were loaded and driven around for 2 h on minor roads with frequent roundabouts and speed bumps, resulting in the truck changing speed and periodic stopping/starting. Meanwhile, pigs of the other two treatments (minimal and high preslaughter stress after short transport) were loaded on a second, larger, but otherwise similar, truck (double-deck truck with all pigs loaded onto the upper deck). After returning to the farm, the two groups of the long transport treatment were unloaded and reloaded onto the second truck, and transported together with the two groups of pigs of the short transport treatment to the processing plant. This transport was mostly smooth (paved, four-lane highways), and took approximately 50 min. After arrival, pigs were kept within their treatments in seperate holding pens at a stocking density of 0.75 m^2/100 kg BW. Pigs in the long lairage treatment received a 3-h rest before slaughter, whereas pigs that were subjected to the short lairage treatment were only rested for 30 to 45 min. Experimental pigs were the first to be slaughtered at 0630, which meant that for the most part lights were dimmed in lairage and there was little noise. All pigs were showered for approximately 10 min directly before they were led to the stunning area. Experimental stressor treatments were the same as described by Hambrecht et al. (2004b). Pigs in the minimal stress group were guided to the stunning area without the use of electric goads and were handled as calmly as possible, whereas pigs in the high stressor treatments were forced, by yells and electric goads, to move four times back and forth in the corridor leading to the stunning area. Pigs were electrically stunned in a fully automated, head-to-heart stunning system (MIDAS, Stork, The Netherlands), and care was taken that all pigs were shackled using the right hind leg.

Sampling procedures and chemical analyses

At 135 min postmortem, when carcasses had passed from the rapid chilling tunnel into the cooler, muscle samples were taken from the longissimus muscle (**LM**) at the last rib and at 160 min postmortem another sample was taken from the supraspinatus muscle (**SSP**) for determination of the glycolytic potential. Samples were immediately frozen in liquid nitrogen and stored at -80°C until further analysis. Muscle lactate and glycogen

were extracted by homogenizing the samples in 0.85 *M* perchloric acid. After centrifuging the suspension for 10 min at 1,500 × *g*, the supernatant was neutralized with 10 *M* KOH. Lactate in the supernatant was determined spectrophotometrically with lactate dehydrogenase and NAD (Bergmeyer, 1974). Glycogen in the supernatant was enzymatically hydrolysed to glucose by incubation with amyloglucosidase (Sigma A 7420; Sigma-Aldrich Chemie BV, Zwijndrecht, The Netherlands) in acetate buffer (pH 4.8) at 55°C for 2 h. After incubation, the supernatant was neutralized with 10 *M* KOH, and glucose was determined spectrophotometrically by the hexokinase method (Bergmeyer, 1974). The concentrations of glucose-6-phosphate and glucose were not separately determined but are included in the glycogen determination. The glycolytic potential was calculated as the sum of 2 × [glycogen] + [lactate] (Monin and Sellier, 1985).

Measurements

At 23 h postmortem various cuts of the left carcass side were harvested for final meat quality assessment. Measurements were taken in the LM at the level of the third lumbar vertebra and in the center of the serratus ventralis muscle (**SV**). Ultimate pH was measured using a portable pH meter (Portamess 911 pH; Knick Elektronische Messgeräte, Berlin, Germany) equipped with a probe-type glass electrode (LoT406; Mettler Toledo, Switzerland). Electrical conductivity was measured using the LF-Star (Ingenieurbüro Matthäus, Nobitz, Germany). Objective color (L*, a*, and b*) was measured on a freshly cut surface after a 10-min blooming period with a Minolta Portable Chroma Meter (Model CR 210; Minolta, Osaka, Japan) equipped with a 50-mm aperture and using illuminant D65.

Statistical analysis

Data were analyzed by the mixed-model procedure (PROC MIXED) of SAS (version 8.02; SAS Inst., Inc., Cary, NC). Least squares means were generated by the LSMEANS statement. Tests of multiple comparisons of LSMEANS were adjusted according to the TUKEY-KRAMER method to ensure the overall significance level of $P = 0.05$. The model applied included the fixed effects of muscle type, transport conditions, lairage duration, and stressor level, as well as their 2-way interactions, and the random effect of slaughter day nested within lairage.

Results

Results of the muscle metabolites and meat quality measurements for the two muscle types are presented in Table 1. In Table 2, effects of the various preslaughter treatments on muscle metabolites and pork quality attributes are presented separately per muscle type. In the LM, representing a glycolytic muscle type, both muscle metabolites and pork quality attributes were measured. Because the SV was not accessible for sampling

in the intact carcass side, for the oxidative muscle type, metabolites were measured in the SSP and pork quality attributes in the SV muscle.

Muscle type affected all traits. Electrical conductivity, however, was similar for both the LM and the SV muscle. The LM was associated with a higher glycolytic potential, an increased lactate formation during the first 135 min postmortem and a higher residual glycogen level compared with the oxidative SSP muscle ($P < 0.001$). Regarding pork quality attributes, the LM was paler (higher L* value), less red (lower a* value), and more yellow (higher b* value) and exhibited a lower ultimate pH than the SV muscle ($P < 0.001$).

The transport treatment had a similar effect on both muscle types. Increasing the length and roughness of transport resulted in an increased ($P < 0.01$) glycolytic potential due to an increased ($P < 0.001$) lactate production but residual glycogen levels were unaffected ($P > 0.05$). Pork of the long and rough transport group was both less red ($P < 0.01$) and less yellow ($P < 0.001$). The lightness of both the LM and the SV muscle was only affected by the transport treatment when lairage duration was short (transport × lairage interaction; $P < 0.05$); long transport compared with short transport resulted then in a darker ($P < 0.05$) color (results not shown). Independent from muscle type or any other treatment, the long transport treatment decreased water-holding capacity as indicated by increased ($P < 0.05$) electrical conductivity values. Ultimate pH tended ($P = 0.077$) to be higher after long compared with short transport.

Decreasing the duration of lairage from 3 h to less than 45 min tended to decrease both the glycolytic potential ($P = 0.083$) and residual glycogen levels ($P = 0.063$). For lactate formation, on the other hand, there was a lairage × muscle interaction ($P < 0.05$). Short lairage duration had no effect on the LM, whereas in the SSP muscle there was a lower ($P < 0.05$) lactate content. Short compared with long lairage led to a darker ($P < 0.01$), less red ($P < 0.05$) and less yellow ($P < 0.05$) pork color. The decrease in redness appeared to be larger in the SV than in the LM (lairage × muscle interaction; $P < 0.05$). Lairage did not affect ($P > 0.05$) ultimate pH. Regarding electrical conductivity, however, lairage affected the response to the high stressor treatment; short as opposed to long lairage resulted in both the LM and the SV muscle in a, numerically, larger stress-induced increase in electrical conductivity (lairage × stress interaction; $P < 0.05$; results not shown).

Table 1. Least squares means (LSmeans) and SE for muscle metabolites in the longissimus (LM) and the supraspinatus (SSP) muscles with *P*-values of the effects of muscle type and preslaughter treatment (short (50 min) and smooth vs. long (3 h) and rough transport, long (3 h) vs. short (< 45 min) lairage, and minimal vs. high stressor level) as well as the muscle type × treatment interactions

	LSmeans			*P*-values						
Muscle:	LM	SSP	Pooled SE	Muscle (M)	Transport (T)	Lairage (L)	Stress (S)	T × M	L × M	S × M
Number of carcasses	358	358	--	--	--	--	--	--	--	--
GP, μmol/g[a]	130.9	57.4	1.11	**0.001**	**0.002**	0.083	**0.001**	0.624	0.240	0.973
Lactate, μmol/g	72.3	50.9	0.79	**0.001**	**0.001**	0.204	**0.001**	0.769	**0.019**	**0.001**
Glycogen, μmol/g[b]	29.3	3.3	0.41	**0.001**	0.854	0.063	**0.001**	0.456	0.426	**0.001**

[a]Glycolytic potential, sum of 2 × [glycogen] + [lactate].
[b]Includes glucose and glucose-6-phosphate concentrations.

Table 2. Least squares means (LSmeans) and SE for pork quality attributes in the longissimus (LM) and the serratus ventralis (SV) muscles with *P*-values of the effects of muscle type and preslaughter treatment (short (50 min) and smooth vs. long (3 h) and rough transport, long (3 h) vs. short (< 45 min) lairage, and minimal vs. high stressor level) as well as the muscle type × treatment interactions

	LSmeans			*P*-values						
Muscle:	LM	SV	Pooled SE	Muscle (M)	Transport (T)	Lairage (L)	Stress (S)	T × M	L × M	S × M
Number of carcasses[a]	358	358	--	--	--	--	--	--	--	--
L* value[bc]	53.8	38.7	0.12	**0.001**	0.110	**0.008**	**0.001**	0.571	0.211	0.142
a* value[b]	19.2	24.2	0.09	**0.001**	**0.002**	**0.029**	**0.006**	0.300	**0.036**	**0.001**
b* value[b]	5.4	4.2	0.07	**0.001**	**0.001**	**0.042**	**0.001**	0.800	0.963	0.147
24-h (ultimate) pH	5.56	6.12	0.020	**0.001**	0.077	0.493	**0.001**	0.097	0.884	**0.001**
EC, mS[de]	8.3	8.5	0.27	0.349	0.039	0.286	**0.001**	0.609	0.999	**0.001**

[a]For 24-h pH, n = 322 and 336 for the LM and SV, respectively.

[b]L* = a measure of darkness to lightness (higher value indicates a lighter color); a* = a measure of redness (higher value indicates a redder color); b* = a measure of yellowness (higher value indicates a more yellow color).

[c]Transport × lairage interaction ($P = 0.038$).

[d]EC = electrical conductivity (higher value indicates a lower water-holding capacity).

[e]Lairage × stress interaction ($P = 0.017$).

Table 3. Least squares means for muscle metabolites measured in the longissimus (LM) and the supraspinatus (SSP) muscles as affected by preslaughter treatment (short (50 min) and smooth vs. long (3 h) and rough transport, long (3 h) vs. short (< 45 min) lairage, and minimal vs. high stressor level) and muscle (LM and SSP)[a]

	Transport × muscle				Lairage × muscle				Stress × muscle			
Muscle:	LM		SSP		LM		SSP		LM		SSP	
Transport:	Short	Long	Short	Long								
Lairage:					Long	Short	Long	Short				
Stress:									Minimal	High	Minimal	High
No. of carcasses	174	184	174	184	179	179	179	179	177	181	177	181
GP, μmol/g[b]	129.4^{z}	132.5^{z}	55.3^{x}	59.5^{y}	132.2^{y}	129.7^{y}	60.0^{x}	54.8^{x}	134.1^{z}	127.8^{y}	60.6$_{x}$	54.3^{w}
Lactate, μmol/g	70.2^{y}	74.4^{z}	49.1^{w}	52.7^{x}	**72.1^{z}**	**72.6^{z}**	**52.9^{y}**	**48.9^{x}**	**58.1^{y}**	**86.5^{z}**	**51.2^{x}**	**50.6^{x}**
Glycogen, μmol/g[c]	29.6^{y}	29.1^{y}	3.1^{x}	3.4^{x}	30.1^{y}	28.6^{y}	3.6^{x}	3.0^{x}	**38.0^{z}**	**20.7^{y}**	**4.7^{x}**	**1.8^{w}**

[a]Where $P < 0.05$ for a preslaughter treatment × muscle interaction, results are printed in bold.

[b]Glycolytic potential, sum of 2 × [glycogen] + [lactate].

[c]Includes glucose and glucose-6-phosphate concentrations.

Table 4. Least squares means for pork quality attributes in the longissimus (LM) and the serratus ventralis (SV) muscles as affected by preslaughter treatment (short (50 min) and smooth vs. long (3 h) and rough transport, long (3 h) vs. short (< 45 min) lairage, and minimal vs. high stressor level) and muscle (LM and SV)[a]

	Transport × muscle				Lairage × muscle				Stress × muscle			
Muscle:	LM		SSP		LM		SSP		LM		SSP	
Transport:	Short	Long	Short	Long								
Lairage:					Long	Short	Long	Short				
Stress:									Minimal	High	Minimal	High
No. of carcasses[b]	174	184	174	184	179	179	179	179	177	181	177	181
L* value[c]	53.9^{y}	53.7^{y}	38.9^{x}	38.5^{x}	54.3^{z}	53.3^{y}	39.4^{x}	38.0^{w}	53.9^{z}	53.7^{z}	39.1^{y}	38.3^{x}
a* value[c]	19.4^{y}	19.1^{x}	24.3^{z}	24.1^{z}	**19.4^{x}**	**19.1^{x}**	**24.5^{z}**	**23.9^{y}**	**19.2^{x}**	**19.3^{x}**	**24.5^{z}**	**24.0^{y}**
b* value[c]	5.5^{z}	5.3^{y}	4.3^{x}	4.1^{x}	5.5^{y}	5.2^{y}	4.3^{x}	4.0^{x}	5.5^{z}	5.2^{y}	4.4^{x}	4.0^{w}
24-h pH (ultimate)	5.55^{x}	5.56^{x}	6.08^{y}	6.14^{y}	5.54^{x}	5.57^{x}	6.10^{y}	6.12^{y}	**5.52^{w}**	**5.59^{x}**	**6.01^{y}**	**6.22^{z}**
EC, mS[d]	8.0	8.5	8.3	8.6	8.0	8.6	8.2	8.8	**6.4^{w}**	**10.2^{z}**	**7.6^{x}**	**9.3^{y}**

[a]Where $P < 0.05$ for a preslaughter treatment × muscle interaction, results are printed in bold.

[b]Glycolytic potential, sum of 2 × [glycogen] + [lactate].

[b]For 24-h pH, n = 157, 165, 163, 173, 177, 145, 179, 157, 163, 159, 177, and 159, respectively.

[c]L* = a measure of darkness to lightness (higher value indicates a lighter color); a* = a measure of redness (higher value indicates a redder color); b* = a measure of yellowness (higher value indicates a more yellow color).

[d]EC = electrical conductivity (higher value indicates a lower water-holding capacity).

[w,x,y,z]Least squares means, within a two-way interaction, lacking a common superscript letter differ ($P < 0.05$).

Stressor level affected all traits that were measured but many of the effects depended on the type of muscle. In both muscle types, glycolytic potential was decreased ($P < 0.001$) by the high stressor treatment. The decrease in residual glycogen in the LM was larger than in the SSP (stress × muscle interaction; $P < 0.001$). In the LM, the reduced glycogen levels were reflected in an increased lactate formation but there was no effect on lactate formation in the SSP (stress × muscle interaction; $P < 0.001$). Independent from muscle type, pork color was darker ($P < 0.001$) and less yellow ($P < 0.001$) after high compared with minimal preslaughter stress. The redness of the pork was unaffected in the LM but in the SV muscle, redness was decreased ($P < 0.01$) by the high stressor treatment (stress × muscle interaction; $P < 0.001$). In both the LM and SV muscle, there was an increase ($P < 0.05$) in ultimate pH noted but this increase appeared to be larger in the SV (stress × muscle interaction; $P < 0.001$). In contrast, electrical conductivity was more increased in the LM than in the SV (stress × muscle interaction; $P < 0.001$). Moreover, the minimal stressor treatment resulted in the LM in the lowest conductivity value compared with all other measurements. Whereas, the high stressor treatment, in the same muscle, caused a higher electrical conductivity than the minimal stressor treatment in both the LM and SV as well as the high stressor treatment in the SV.

Discussion

In the present experiment, the LM and SV muscle were chosen for pork quality assessment based on their metabolic and contractile properties, economical importance, and liability to quality problems. Muscle glycolytic potential was measured in the LM, predominantly containing fast-twitch glycolytic fibers, and the SSP, a muscle of the shoulder containing similar to the SV a high portion of slow-twitch oxidative muscle fibers (Ruusunen et al. 1988; Warriss et al., 1990; Bee et al., 1999). For simplicity reasons, the SV and SSP muscles will be referred to as "oxidative" whereas the LM will be referred to as "glycolytic". The SSP muscle was chosen for determination of glycolytic potential because, as opposed to the SV muscle, this muscle was accessible for sampling in the intact carcass side early postmortem.

Few studies have assessed both glycolytic potential and pork quality beyond ultimate pH in muscles other than the LM. Generally, differences in the present experiment between the LM and the SSP and the SV muscles, respectively, are in agreement with several studies showing for oxidative compared with glycolytic muscles a lower glycolytic potential (Monin et al., 1987; Fernandez et al., 1994), a darker color (Warner et al., 1993; Brewer et al., 2001), and a higher ultimate pH (Barton-Gade and Olsen, 1987; Monin et al, 1987; Warner et al., 1993). Warner et al. (1993) additionally reported a lower exudate for oxidative compared with glycolytic muscle which is in disagreement with the similar electrical conductivity values, a measure for water-holding capacity, that were found in the present study for both the SV and the LM. However, Warner et al. (1993) selected carcasses that exhibited a wide range of quality, with more than 20%

having an initial pH lower than 5.8 whereas this number was less than 1% in the present study (results not shown).

Focus of the present study were the differences in the responses to preslaughter handling treatments rather than the differences between the two different muscle types itself. The transport, lairage, and stress treatments were chosen to result in various psychological and physical stress levels (Bradshaw et al., 1996, Pérez et al., 2002b, Hambrecht et al., 2004b) that are frequently encountered in commercial practice. Detailed results of the effects of transport, lairage, and preslaughter stress level on stress indicators, LM glycolytic potential, and LM quality, as well as their interactions, are described and discussed elsewhere (Hambrecht et al., unpublished).

Effects of transport

The physical exercise and psychological stress that is associated with transport varies depending on the type of transport. Transport in general was shown to be stressful for pigs (Becker et al., 1985; Geverink et al., 1998) and even more so in rough as opposed to smooth transports (Bradshaw et al., 1996). As a consequence, both the physical exercise and the psychological stress level was probably higher in the long and rough compared with the short and smooth transport treatment. Oxidative muscle fibers are preferentially recruited at low intensity exercise levels (Køpke et al., 1984; Lacourt and Tarrant, 1985). Because the physical exercise level associated with transport is probably low, the long and rough transport treatment was expected to reduce glycogen to a larger extent in a oxidative as opposed to a glycolytic muscle, as was shown in sheep by Monin and Gire (1980). In the present study, however, both the SSP and the LM reacted similarly to the transport treatments. Moreover, no glycogen depletion was noted for the longer transport but, instead, an increased lactate formation that resulted in an increased glycolytic potential. This is in apparent contradiction with the ultimate pH values that tended to be increased after long transport. Karlsson et al. (1994) showed for pigs with similar glycogen levels differences in ultimate pH. However, the amount of glycogen depleted fibers was different and therefore they suggested that not glycogen level alone but also glycogen distribution within a muscle affects pork quality.

Effects of lairage

Lairage is not a stress factor itself but is meant to provide a rest for pigs to recover from previous transport stress. Prolonged lairage may promote fighting among pigs and lead to energy depletion (Warriss, 2003). The tendency towards a slight increase in muscle glycogen level in the present study indicates that pigs indeed rested during lairage. There are some indications that glycogen replenishment during recovery from physical exercise depends on muscle fiber type (in rats: Górski et al., 1978; McLane and Holloszy, 1979; in man: Fairchild et al., 2003). In the present study, however, no differences in glycogen repletion during lairage were observed between the LM and

SSP muscle. The exercise level associated with either of the two transport treatments was probably too low to induce different patterns of glycogen depletion and replenishment in muscles differing in metabolic type. Effects on pork quality were probably mediated by differences in the psychological stress level and not by differences in physical activity associated with the various transport and lairage combinations. Stress induces the secretion of, among other hormones, epinephrine that can lead to both glycogen depletion and an increased glycolysis (Fernandez et al., 1995a; Jensen et al., 1999). Oxidative muscle fibers show a better vascularisation and a higher β-adrenergic receptor density (Essén-Gustavsson et al., 1992; Jensen et al., 1995) and are therefore believed to be more sensitive to epinephrine. But similar to the exercise intensity, psychological stress levels related to the transport and lairage treatments were probably too low to exert different effects on the two muscle types.

Effects of stress

The high stressor treatment was previously tested and was shown to be associated with a high level of both physical exercise and psychological stress and large effects on pork quality (Hambrecht et al. 2004a,b,unpublished). In agreement with the previously mentioned differences in epinephrine sensitivity and responses to exercise intensity, effects of the stressor treatment depended on the muscle type. However, although only in the LM a large increase in lactate formation was noted, it cannot be concluded that the high stressor treatment did not lead to a higher rate of lactate formation in the oxidative muscles. At 160 min postmortem, when SSP muscle samples were taken, almost the entire SSP glycogen stores were converted into lactate. To assess whether lactate production rate was also increased in the SSP in response to high stress, muscle samples should have been taken at an earlier moment. This is supported by electrical conductivity values that were increased in both the LM and the SV. The increase was more than twice as large in the LM (+ 59.4%) compared with the SV (+ 22.4%). The SV muscle, on the other hand, was more sensitive to high preslaughter stress regarding pork color. Additionally, the SV muscle showed a larger increase in ultimate pH compared with the LM. This is in agreement with studies showing a larger pH-increasing effect of preslaughter stress on oxidative compared with glycolytic muscles (Warriss et al., 1995; Brown et al., 1998). Barton-Gade and Olsen (1987) compared stress-susceptible pigs with stress-resistant pigs and found an increased incidence of PSE in glycolytic muscles such as the LM whereas oxidative muscles, including the SV, showed an increased incidence of the DFD condition. These results are supported by the effects of the high-stressor treatment in the present experiment.

Implications

Increasing duration and roughness of transport as well as a short lairage time affected both the glycolytic longissimus muscle (loin) and the oxidative SSP and SV (both muscles of the shoulder) in a similar way. However, the considerably higher physical

and psychological stress level associated with the high stressor treatment immediately before slaughter decreased the water-holding capacity to a larger extent in the LM than in the SV. Effects on color and ultimate pH, on the other hand were larger in the SV muscle. High stress levels associated with preslaughter handling may promote PSE development in predominantly glycolytic muscles whereas oxidative muscles will rather develop DFD pork.

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Effects of various preslaughter handling treatments on the color of different pork muscles

6A

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Abstract

The objective of the present experiment was to investigate whether the effects of transport, lairage, and preslaughter stress on color are different in various pork muscles. Commercial, halothane-free pigs (n = 384) were assigned to one of eight treatments in a 2 × 2 × 2 factorial arrangement, with two types of transport (short (50 min) and smooth or long (3 h) and rough), two lairage durations (long (3 h) or short (<45 min) and two stress levels immediately before slaughter (minimal or high). Eight groups of 48 pigs were processed on various days at a commercial plant. At 23 h post-mortem pork cuts were harvested for color measurements. L* (lightness), a* (redness), and b* (yellowness) values were assessed in the predominantly glycolytic longissimus lumborum (**LL**), longissimus thoracic (**LT**), and semimembranosus (**SM**) muscles as well as in the predominantly oxidative serratus ventralis muscle (**SV**). Transport affected the various muscles in a similar way. The effects of the lairage treatments, on the other hand, depended on the muscle type. L* values were decreased ($P < 0.05$) in the SV muscle, whereas the response was small in the LT and intermediate in the SM and LL muscles (lairage × muscle interaction; $P < 0.05$). The SV was the only muscle that showed a decreased ($P < 0.05$) redness after short lairage (lairage × muscle interaction; $P < 0.05$). Similar to lairage, the effects of the stressor treatments were different in the various muscles. High stress compared with minimal stress resulted in paler meat ($P < 0.05$) in the LT whereas the SV muscle became darker ($P > 0.05$; stress × muscle interaction; $P < 0.001$). Meat was less red ($P < 0.05$) in both the LT and the SV for the high stressor treatment while SM redness seemed to be increased ($P > 0.05$) by high compared with minimal stress (stress × muscle interaction; $P < 0.001$). No effect on yellowness was seen in the SM but yellowness was increased ($P < 0.05$) in the LT and decreased ($P < 0.05$) in both the LL and the SV muscles in response to high stress (stress × muscle interaction; $P < 0.001$). It is concluded that effects of preslaughter handling depend on the muscle that is studied. High stress levels in the preslaughter period may promote PSE development in glycolytic muscles whereas more oxidative muscles will rather develop DFD pork. Effects of preslaughter handling on pork quality should be assessed not only in one but in several muscles.

Introduction

Color is one of the most important quality attributes of pork because color can be easily assessed by both producers and consumers. In addition, color is related to other important quality aspects. Various phases during preslaughter handling, such as transport (Leheska et al., 2003), lairage (Pérez et al., 2002) and stress immediately before slaughter (Hambrecht et al., 2004b) may affect pork color. Due to its size and accessibility, the LM is the most frequently assessed muscle. However, muscles consist of various fiber types that determine the metabolic properties of a muscle. It can be expected that muscles, depending on their anatomical location and metabolic profile, react differently to the physical and psychological stressors associated with preslaughter handling. In agreement, Warner et al. (1993) found that the longissimus, a predominantly glycolytic muscle, was a reliable pork quality indicator for other muscles when its color was dark and exudate low. However, when its color was pale and exudate high, quality of the longissimus muscle was only related to the major ham muscles and not to the shoulder muscles that have a more oxidative metabolism. The objective of the present experiment was to investigate whether the effects of transport, lairage, and preslaughter stress on color are different in various, commercially important pork muscles.

Materials and methods

All pigs were commercial halothane-free end products of the *Hypor* pig breeding company. Pigs (n = 384) were assigned to one of eight treatments in a 2 × 2 × 2 factorial arrangement, with two types of transport (short (50 min) and smooth or long (3 h) and rough), two lairage durations (long (3 h; considered as optimal) or short (<45 min; considered as sub-optimal)) and two stress levels immediately before slaughter (minimal or high). Eight groups of 48 pigs, all originating from the same commercial farm, were processed during eight weeks on various days. Long and short lairage alternated between consecutive weeks. Transport types and preslaughter stress levels were varied within the same slaughter day. Pigs were electrically stunned in a fully automated, head-to-heart stunning system at a commercial plant. At 23 h post-mortem pork cuts were harvested for color measurements. All measurements were done after a 10-min blooming period with a Minolta Portable Chroma Meter (Model CR 210). L* (lightness), a* (redness), and b* (yellowness) values were assessed in the longissimus lumborum muscle (**LL**) at the level of the third lumbar vertebra, in the longissimus thoracic muscle (**LT**) at the level of the 6th thoracic vertebra, in the serratus ventralis muscle (**SV**), and in the semimembranosus muscle (**SM**). Data were analysed by the mixed-model procedure (PROC MIXED) of SAS. Tests of multiple comparisons of least squares means were adjusted according to the TUKEY-KRAMER method to ensure the overall significance level of P = 0.05. The model applied included the fixed effects of

muscle type, transport conditions, lairage duration, and stressor level, as well as their 2-way interactions, and the random effect of slaughter day nested within lairage.

Results and discussion

Effects of transport

Results are presented in Table 1. Long and rough compared with short and smooth transport decreased ($P < 0.05$) the yellowness of the meat. No other main effects or interactions between the transport treatment and muscle type were observed. The meat was darker (i.e. lower L* values) when transport was long and rough and pigs were additionally subjected to the short lairage treatment (transport × lairage interaction; $P < 0.05$; results not shown). This effect was similar for all muscles but the largest effect was observed in the SV muscle. Transport had no effect on the redness in the minimal preslaughter stress group. In the high stress group, however, long as opposed to short transport increased the redness in the LT and SM muscles but decreased the redness in the SV muscle ($P < 0.05$; results not shown). LL redness was not affected ($P > 0.05$). These effects on redness are probably related to differences in the relative proportions of the various myoglobin forms (Lindahl et al., 2001) but remain difficult to explain. The physical exercise and psychological stress that is associated with transport varies depending on the type of transport. Transport in general was shown to be stressful for pigs (Geverink et al., 1998) and even more so in rough as opposed to smooth transports (Bradshaw et al., 1996). As a consequence, both the physical exercise and the psychological stress level was probably higher in the long and rough compared with the short and smooth transport treatment. Oxidative muscle fibers are preferentially recruited at low intensity exercise levels (Køpke et al., 1984; Lacourt and Tarrant, 1985) and were shown to be more sensitive to adrenaline (Górski, 1978; Fernandez et al., 1995) which is secreted in response to psychological stress. Consequently, it was expected that the SV, a more oxidative muscle compared with the other muscles, would show a larger response. This was only partly true, which is probably related to the small differences in exercise and stress level between the two transport treatments.

Table 1. Effect of transport, lairage, and preslaughter stress on pork color in the longissimus lumborum (LL), the longissimus thoracic (LT), the semimembranosus (SM), and the serratus ventralis (SV) muscles[a]

		L* value		a* value		b* value	
Transport × muscle	Transport:	Short	Long	Short	Long	Short	Long
	LL	53.9	53.7	19.4	19.1	5.5	5.3
	LT	58.9	59.1	19.2	19.2	5.6	5.6
	SM	54.4	54.0	19.7	19.9	6.5	6.5
	SV	38.9	38.5	24.3	24.1	4.3	4.1
	SE_{pooled}	0.23	0.23	0.09	0.09	0.10	0.10
Lairage × muscle	Lairage:	Long	Short	Long	Short	Long	Short
	LL	54.4	53.0	19.4	19.1	5.5	5.2
	LT	59.2	58.9	19.3	19.1	5.6	5.6
	SM	54.5	53.6	19.6	19.9	6.7	6.3
	SV	39.4^{y}	37.7^{x}	24.5^{y}	23.9^{x}	4.3	4.0
	SE_{pooled}	0.25	0.25	0.09	0.09	0.12	0.12
Stress × muscle	Stress:	Minimal	High	Minimal	High	Minimal	High
	LL	53.9	53.7	19.2	19.3	5.5^{y}	5.2^{x}
	LT	57.1^{x}	60.8^{y}	19.5^{y}	18.9^{x}	5.3^{y}	5.9^{x}
	SM	54.2	54.2	19.6	20.0	6.5	6.5
	SV	39.1	38.3	24.5^{y}	23.9^{x}	4.4^{y}	4.0^{x}
	SE_{pooled}	0.23	0.23	0.09	0.09	0.10	0.10
P-values	Transport (T)	0.206		0.157		**0.010**	
	Lairage (L)	**0.024**		**0.061**		0.110	
	Stress (S)	**0.001**		**0.002**		0.303	
	Muscle (M)	**0.001**		**0.001**		**0.001**	
	T × M	0.509		0.102		0.589	
	L × M	**0.009**		**0.001**		**0.016**	
	S × M	**0.001**		**0.001**		**0.001**	
	T × L	**0.020**		0.547		0.491	
	T × S	0.411		**0.003**		0.426	
	L × S	0.524		0.736		0.127	

[a]n = 174/184 (short/long transport), 179/179 (long/short lairage), 177/181 (minimal/high stress).
[xy]Least squares means within muscle, 2-way interaction and color attribute lacking a common superscript letter differ ($P < 0.05$).

Effects of lairage

For the lairage treatments there was for all color values an interaction between lairage and muscle type noted ($P < 0.05$). For the L* value, this was probably due to the relatively large decrease ($P < 0.05$) in lightness in the SV muscle, whereas the response to the short lairage duration was small in the LT and intermediate in the SM and LL muscles ($P > 0.05$). Regarding the a* value, the SV was again the only muscle that showed a difference ($P < 0.05$) between long and short lairage with a decreased redness after a short lairage period. None of the pair wise comparisons were significant for the b* values, but both the LL, SM and SV muscles appeared to exhibit a, numerically, lower yellowness for the short lairage treatment whereas there was no difference in b* values noted for the LT. Lairage is not a stress factor itself but is meant to provide a rest for pigs to recover from previous transport stress. Lairage times shorter than 45 min are usually considered as too short for recovery (Milligan et al., 1998; Pérez et al., 2002) whereas 3 h is often regarded as optimal for pigs (Warriss et al. 1998; Pérez et al., 2002). In agreement with the above mentioned higher adrenaline sensitivity and recruitment at low exercise intensity of oxidative muscle fibers, the oxidative SV muscle showed a somewhat larger response to the short lairage treatment. Another reason may be the heavier involvement of the shoulder and neck muscles in activities such as posture holding during transport, exploring and fighting due to mixing of foreign pigs and, as a consequence, an increased need for rest compared with other muscles.

Effects of stress

For all color values, there were interactions between the stressor treatment and muscle ($P < 0.001$). High stress compared with minimal stress resulted in paler meat in the LT ($P < 0.05$) whereas the SV muscle became darker in response to high stress ($P > 0.05$). Meat was less red ($P < 0.05$) in both the LT and the SV for the high stressor treatment while SM redness seemed to be increased ($P > 0.05$). Whereas LL lightness and redness appeared to be hardly affected by preslaughter stress, the yellowness of the LL was, similar to SV yellowness, decreased ($P < 0.05$). No effect on yellowness was seen in the SM but LT yellowness was increased ($P < 0.05$) in response to high stress. The high stressor treatment was previously tested and shown to be associated with a high level of both physical exercise and psychological stress as well as large effects on pork quality (Hambrecht et al. 2004a,b). In agreement with the previously mentioned differences in adrenaline sensitivity, response to exercise intensity and involvement in physical activity, effects of the stressor treatment depended on muscle type. Barton-Gade and Olsen (1987) compared stress-susceptible pigs with stress-resistant pigs and found an increased incidence of PSE in glycolytic muscles such as the LM whereas oxidative muscles, including the SV, showed an increased incidence of the DFD condition. These results are supported by the effects of the high stressor treatment in the present experiment. Additionally, the present study shows that considerable variation exists for pork color measured within the longissimus muscle. The high

stressor treatment produced different and larger effects in the LT compared with the LL muscle. The reasons for these differences within the longissimus muscle are unknown. But results are in agreement with Lundström and Malmfors (1985) who showed a higher incidence of PSE in the shoulder part of the loin, compared with the mid-loin and the ham site of the loin.

Conclusions

Effects of preslaughter treatments depend on the muscle that is studied. The metabolic profile but also the location of a muscle play a crucial role. High stress levels associated with preslaughter handling may promote PSE development in some glycolytic muscles whereas more oxidative muscles will rather develop DFD pork. Effects of preslaughter handling on pork quality should be assessed not only in one but in several muscles.

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General discussion

7

Various pre- and postslaughter factors and their effects on pork quality and to a lesser extent also on animal welfare have been studied in this thesis. The main objective was to identify critical stages in the preslaughter period related to pork quality that can be controlled (at least partly) by processing plants. The intention was not only to improve the level of the water-holding capacity and the color of pork but also to reduce the variation in quality both between and within different groups of slaughter pigs. In this final chapter, the research approach and scope of this thesis are critically reviewed as well as whether and to what extent the objectives have been reached. Following and concluding, the scientific and practical implications of the outcomes are discussed beyond the direct conclusions that are included in the individual preceding chapters.

Research approach and scope of this thesis

Commercial vs. experimental conditions

All studies were conducted at various commercial processing plants, that is all slaughter and processing conditions (line speed, stunning, scalding, evisceration etc.) corresponded to practices at large commercial plants. These commercial circumstances had the consequence that the execution of especially early postmortem handlings such as initial pH measurement or muscle sampling was more difficult than under experimental conditions in research facilities. A large number of people was needed to keep up with the high line speeds (420 to 600 pigs/h) which limited the number of early postmortem measurements that could be accomplished at a specific time around slaughter. Moreover, the moment of measurement and sampling was partly dictated by the inherent strict production routines of large operations. These, from a research point of view, disadvantages were accepted because the direct applicability of the results was one of the main aims of these investigations. There are several reasons why the translation of results obtained under experimental conditions into commercial practice may be difficult which will be elucidated in the following.

Capacity. The capacity at experimental abattoirs is usually low compared with line speeds of several hundred pigs per hour at commercial plants. Therefore, the number of pigs that can be processed on the same day under identical feed withdrawal, transport, and lairage conditions is usually more limited at an experimental abattoir than at a commercial plant. Moreover, in commercial plants, lairage groups are usually larger (approximately 50 pigs; in a survey of Geverink et al., 1996) than in experimental studies (e.g. 5 to 10 pigs in Stalder et al., 1998 and Brown et al., 1998). Mixing of unfamiliar pigs during transport and lairage may induce fighting, resulting in arousal of the pigs and disturbed resting (Warriss and Brown, 1985; Geverink et al., 1998). Geverink et al. (1996) argued that increasing lairage groups may prolong the occurrence of fighting. Consequently, effects of preslaughter handling treatments may be influenced by the group size and the number of unfamiliar pigs during transport and lairage.

Stunning systems. At experimental abattoirs, pigs are usually electrically stunned by the manual application of tongs (e.g. Hartung et al., 1997; van der Wal et al., 1999) or by CO_2 in a dip lift stunner (e.g. Henckel et al., 1998; Channon et al., 2003). In large plants, however, automatic head-to-heart stunning systems may be employed while large-scale CO_2 stunning is mostly performed in paternoster systems or in newly developed group stunning equipments. It is well known that the method of stunning may affect pork quality (Henckel et al., 1998; Channon et al., 2000; Velarde et al., 2000, 2001). But there also exist especially between various electrical stunning systems differences in the effects on pork quality (Channon et al., 2003). Positioning of the tongs, stunning duration, and voltage are characteristics that may significantly influence not only the effectiveness of a stun but also pork quality (van der Wal, 1978; Gregory, 1985; Anil and McKinstrey, 1998). Correct positioning of the tongs and a short stunning duration in combination with the low voltage used to induce cardiac arrest favor automated head-to-heart to manual electrical stunning systems. Comparing various CO_2 stunning facilities, on the other hand, dip lift systems may be better from a welfare point of view and possibly also when looking at meat quality than paternoster systems. Exposure to CO_2 is distressful for pigs (Raj and Gregory, 1995, 1996; Raj, 1999) and immediate exposure to high CO_2 concentrations are desirable because they guarantee a rapid loss of consciousness (Raj and Gregory, 1996). At the first stop in paternoster systems, however, CO_2 concentrations may be lower than at the bottom of the pit so that the immediate lowering into the highest gas concentrations in dip lift systems may be beneficial for both welfare and meat quality. In conclusion, effects of stunning and related handlings studied in experimental abattoirs cannot be translated without caution directly to large commercial operations.

Stress level. Brown et al. (1998) investigated the effects of preslaughter stress under experimental conditions and concluded that it is difficult to simulate commercial treatment adequately under laboratory conditions. In agreement, plasma concentrations of lactate and cortisol, indicators of physical and psychological stress, were consistently higher in our studies (Chapter 4 and 5) compared with studies that took place under experimental conditions (Hartung et al., 1997; Brown et al., 1998). As discussed in Chapter 5, differences in preslaughter handling related to transport and lairage of pigs resulted in an impaired pork quality only in combination with the high stressor treatment. Because similarly high stress levels are hardly encountered under experimental conditions (Brown et al., 1998), the interaction between suboptimal transport and lairage conditions on the one hand and stressor level on the other, might not have been found when studying the same transport and lairage treatments in an experimental abattoir. In other words, lower preslaughter stress levels associated with experimental abattoirs might obscure differences between treatments that are likely to become significant in commercial plants.

Scope of this thesis

The above described aspects together have resulted in the decision to conduct all the experiments within this thesis in commercial plants in order to guarantee the direct applicability. As a logical consequence, the practical relevance of the experimental treatments that were employed in the various studies was an important connecting thread throughout this thesis. The stressor treatments in Chapter 3-6, the chilling regimes in Chapter 3 as well as the transport and lairage conditions in Chapter 5 and 6 were all within limits that may be encountered at commercial processing plants.

Although genetics (Monin and Sellier, 1985; Channon et al., 2000), feed composition (Rosenvold et al., 2001a,2003), feed additives (Asghar et al., 1991; D'Souza et al., 1999), and feed withdrawal time (Murray et al., 2001; Leheska et al., 2003) were shown to influence pork quality to a greater or lesser degree, these factors were merely standardized and not included in the selection of experimental treatments. This fact is related to the objective of this thesis, namely to study exclusively factors that can be controlled by processing plants. Because at least the duration of transport is mostly a given fact and cannot be adapted to the needs of the processing plant, the inclusion of two different transport treatments (Chapter 5 and 6) apparently contradicts this objective. However, the physical and psychological condition in which the pigs arrive at the processing plant possibly affects the minimal amount of time needed to recover as well as the pig's reaction to subsequent handling which was the motivation to study the interactions between transport conditions, lairage duration, and preslaughter stress level.

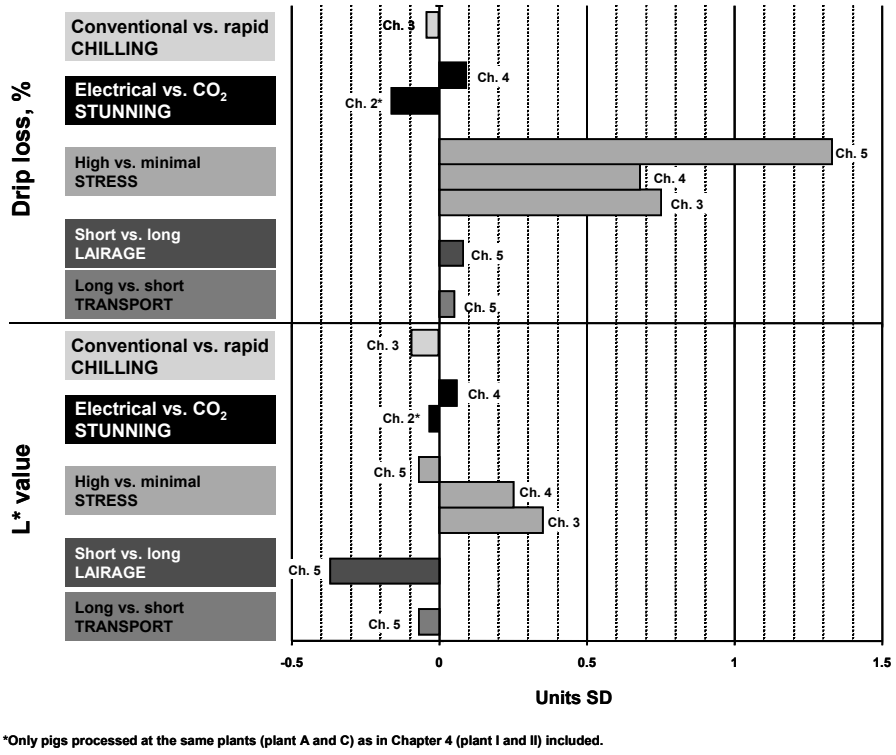
Except for the chilling treatments in Chapter 3, no postmortem measures that have potential to improve pork quality, such as e.g. dehiding instead of scalding (Maribo et al., 1998) or delayed deboning (den Hertog-Meischke et al., 1997) were investigated. Results of Chapter 3 and 4 clearly pointed to preslaughter stress as one of the most crucial factors for both pork quality and animal welfare. In addition, muscle glycogen affected both water-holding properties and pork color (Chapter 4), especially in combination with the high stressor treatment. This warranted the further study of the stressor treatment and its interactions with other handling factors that may influence muscle glycogen and, more importantly, the stress level as experienced by the pigs.

Identification of critical quality points

Results of the study reported in Chapter 2 indicated that despite using pigs of the same genotype and farm origin, pork quality was different at the three plants that were included in the study. The aim of the following studies was to identify critical quality points within or related to the processing plants that were at least partly responsible for the observed differences.

Regarding water-holding capacity, preslaughter stress level clearly exerted the largest impact in all three studies where the stressor treatments were included (Chapter 3-6).

Drip losses were increased by approximately 0.7 to 1.3 standard deviations (SD) by the high compared with the minimal stressor treatment (Figure 1). The effects on the L* value (lightness), which is closely related to visually perceived color (Brewer et al., 2001), were not consistent over studies: stress affected the lightness of the sirloin in the studies reported in Chapter 3 and 4 but not in the study reported in Chapter 5 (Figure 1). In Chapter 2, it was suggested that the method of stunning may partly explain differences in meat quality between the processing plants. The two stunning systems that were employed, a fully automated head-to-heart stunning system (plant A and B) and a paternoster CO_2 stunning system (plant C), could not be directly compared. Nevertheless, results of the plant comparison reported in Chapter 2, where the pork produced at plant C was of slightly inferior quality compared with both plants A and B, were reversed by the outcomes of the study documented in Chapter 4 (Figure 1).



*Only pigs processed at the same plants (plant A and C) as in Chapter 4 (plant I and II) included.

Figure 1. Effects of various pre- and postslaughter factors on drip losses and pork color (L* value; higher values indicate a lighter color). Within treatments, absolute differences between means were divided by the pooled standard deviation (**SD**) for the respective attribute. Letters adjacent to the bars refer to the corresponding Chapter (Ch.).

In this study, no differences were found between plants or, for few attributes, a slightly favorable quality at the plant employing CO_2 stunning. Results of the latter study (Chapter 4) are in agreement with experiments reporting a positive effect on pork quality of CO_2 compared with electrical stunning (Henckel et al., 1998; Channon et al., 2000; Velarde et al., 2000, 2001). However, the effects of the different stunning methods or, more precisely, between the plants employing the different stunning methods reported in this thesis were very small compared with the effects that the stressor treatments had. Chilling, similarly, had a very limited effect on both color and water-holding capacity (Figure 1; Chapter 3). It is very likely that differences in preslaughter stress, though not deliberately inflicted upon the pigs, were responsible for the differences in water-holding properties between the processing plants that are reported in Chapter 2.

Although suboptimal transport and lairage conditions aggravated the negative effects of the high stressor treatment, on its own the effects of transport and lairage were restricted to a slightly darker sirloin color in response to a decreased lairage period (Figure 1). It should be noted that in these studies (Chapter 5, 6, and Appendix to Chapter 6) no excessive transport or lairage durations were included because these conditions were not usual in the three processing plants where the experimental pigs were slaughtered. Long distance transports and overnight lairage may exhaust pigs due to excessively long feed withdrawal times in combination with physical exercise (posture holding during transport, fighting during lairage) and long-term psychological stress, thereby increasing the incidence of DFD meat (Grandin, 1980; Barton-Gade, 1997). Similarly, increasing the lairage duration from 30 min to 3 h, which had a positive effect in the present study in combination with the high stressor treatment, may show adverse effects when environmental temperatures and humidity are high (Fraqueza et al., 1998). As a consequence, care has to be taken when generalizing and extrapolating results regarding the effects of transport and lairage to conditions that greatly differ from the ones studied within this thesis.

In conclusion, results of this thesis strongly suggest that stress in the immediate preslaughter period is the most important single determinant of pork quality that is directly related to and can be controlled by processing plants.

Variation between and within different groups of slaughter pigs

For the pork industry, not only the level but also the uniformity of quality is important. Therefore, it was investigated whether the various factors that were studied within this thesis affect the variation in water-holding capacity (drip losses) and/or pork color (lightness; L* value). In Table 1, means and standard deviations(SD), pooled for the various slaughter groups, are presented for the individual pre- and postslaughter factors.

Table 1. Effect of various pre- and postslaughter factors on the variation in water-holding capacity (drip loss) and pork color (L* value) within and between various slaughter groups

	Factor	n[a]	Treatment				
			Pooled means		Pooled		$\frac{SD_1}{SD_2}$[f]
					SD_1	SD_2	
DRIP LOSS, %			Rapid	Conventional	Rapid	Conventional	
	Chilling[b]	4	1.66	1.65	1.07	0.85	1.26
			CO_2	Electrical	CO_2	Electrical	
	Stunning[c]	13	4.39	4.07	2.10	1.87	1.12
			Minimal	High	Minimal	High	
	Stress[d]	12	0.86	1.56	0.46	0.84	0.55*
			Long	Short	Long	Short	
	Lairage[e]	4	0.94	0.87	0.67	0.63	1.06
			Short	Long	Short	Long	
	Transport[e]	8	0.90	0.92	0.65	0.64	1.02
L* VALUE			Rapid	Conventional	Rapid	Conventional	
	Chilling[b]	4	51.9	51.7	3.27	2.58	1.27
			CO_2	Electrical	CO_2	Electrical	
	Stunning[c]	12	50.4	50.6	3.40	2.89	1.18*
			Minimal	High	Minimal	High	
	Stress[d]	11	53.2	53.4	2.39	3.07	0.78*
			Long	Short	Long	Short	
	Lairage[e]	4	53.3	54.3	2.58	2.80	0.92
			Short	Long	Short	Long	
	Transport[e]	8	53.9	53.7	2.66	2.72	0.98

[a]n = number of slaughter groups; each group consisted of 24 to 48 pigs.
[b]Based on Chapter 3.
[c]Based on Chapter 2 and 4. The higher drip loss values and the larger SD are related to a different drip loss method that was employed in Chapter 2 but not in subsequent Chapters.
[d]Based on Chapter 3, 4, and 5; only data of pigs processed at the same plant (plant I in Chapter 4) were included.
[e]Based on Chapter 5.
[f]Values close to 1 indicate similar standard deviations (SD) of the two respective treatments.
*F-test revealed significant differences between SDs of two treatments ($P < 0.05$).

Transport and lairage had no effect ($P > 0.05$) on the variation in drip losses nor in L* values. Rapid chilling, on the other hand, seemed to increase, numerically, the variation in both drip loss and L* value ($P > 0.05$). However, the standard deviations differed considerably between the various slaughter groups, which probably, in combination

with the low number of slaughter groups (n = 4), is the reason why the relatively large numerical differences between rapid and conventional chilling are statistically not significant.

CO_2 compared with electrical stunning, or, more correctly, plant C vs. plant A and plant II vs. plant I (Chapter 2 and 4, respectively), increased ($P < 0.05$) the variation in L* value but did not affect ($P > 0.05$) the variation in drip loss. In literature, there is little information available on the variation that is caused by a specific treatment. Therefore it is not known whether CO_2 stunning generally causes a larger variation in pork color or whether this effect is restricted to the plant that was studied in this thesis. Because the method of stunning was confounded with processing plant, factors other than the method of stunning may have caused the larger variation.

In Chapter 4, it was reported that blood lactate and glycolytic potential together explained 48% of the variation encountered in L* values. In agreement with the larger variation in L* values, the variation for muscle glycolytic potential (only measured in Chapter 4) was indeed numerically larger at the plant employing CO_2 stunning than at the plant employing electrical stunning (pooled SD 12.8 vs. 10.5). However, differences were statistically not significant ($P > 0.05$). More data are needed to confirm and to explain the effect of stunning method on the variation in pork color.

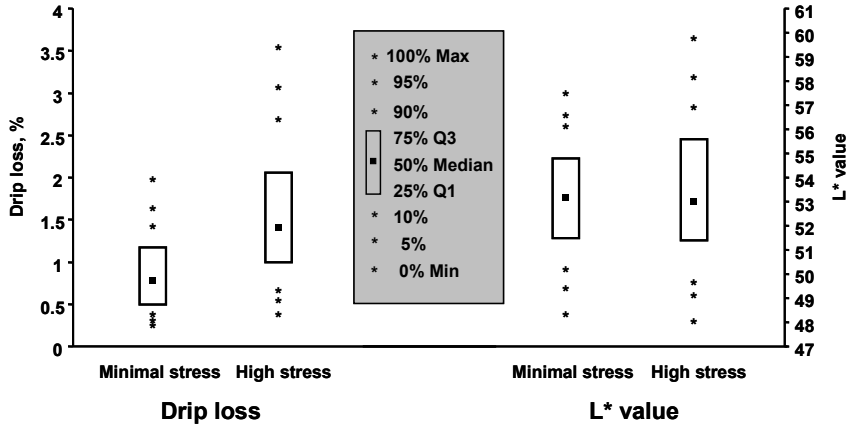


Figure 2 . Effect of stressor level on the variation in water-holding capacity (drip loss) and lightness (L* value) of pork. The graph shows the median (within the boxes) with the lower and upper borders of the boxes representing the 25 and 75% quartiles, respectively. Asteriks indicate in consecutive order the 0, 5, 10, 90, 95, and 100% limits. Based on data from Chapter 4 and 5; only data of pigs processed at the same plant (plant I in Chapter 4) were included.

High compared with minimal stress in the immediate preslaughter period, on the other hand, clearly and consistently increased the variation in both drip losses and L* values (Table 1; Figure 2). This increased variation was accompanied - or, better said, caused - by a similar increase in the variation in blood lactate (pooled SD 4.49 vs. 5.58 for minimal vs. high stress, respectively; $P < 0.05$). Warriss et al. (1994) compared various commercial processing plants and also found the variation in blood lactate increased for the plants associated with 'high stress' compared with 'low stress' plants. However, they did not found a concomitant increase in electrical conductivity, an indicator of water-holding capacity. The stressor treatments that were employed in Chapter 3-6 involved both psychological and physical stress. Individuals show considerable variation in behavioral and endocrine responsiveness in coping with stress (reviewed by Koolhaas et al., 1999). Moreover, Steinhardt et al. (1978) showed both a higher level and a larger variation in blood lactate in response to physical exercise in pigs with a low, compared with pigs with a high endurance capacity. Consequently, differences in the capacity to cope with physical and psychological stressors between individual pigs are probably the main reason for the increased variation in both blood lactate and pork quality after high compared with minimal preslaughter stress.

Scientific and practical implications

Stress assessment

In the previous paragraphs, it has been emphatically stated that the stressor level in the immediate preslaughter period is key for pork quality. Moreover, when this stressor level is high, also preceding handling factors such as transport and lairage may affect pork quality. In addition to improving pork quality, reducing stress in the entire preslaughter period would greatly benefit animal welfare. To some extent, pork quality measurements can be used to assess the success of measures to reduce preslaughter stress. However, variables that are more closely linked to an animal's response to stress are necessary for several reasons.

Firstly, although pork quality and animal welfare are related to each other, changes in one aspect are not necessarily related to similar changes in the other. An improved pork quality is not automatically related to an improved animal welfare. For example, Leheska et al. (2003) concluded that a prolonged feed withdrawal time of 48 h and a transport of 8 compared with 0.5 or 2.5 h resulted in an improved pork quality in terms of a darker loin color, reduced drip losses, and an increased final pH. Obviously, these treatments undermine animal welfare which objectively can be assessed only by the measurement of variables directly linked to the stress response. (Note: Results presented in Chapter 6 and in the Appendix of Chapter 6 suggest that these, for the quality of the loin, beneficial treatments probably resulted in an undesirable increase in the incidence of DFD meat in other pork cuts such as the Boston Butt, which were not assessed in the study of Leheska et al. (2003).)

Secondly, a major disadvantage of pork quality measurements is that they become available only the day after slaughter. On the one hand, this demands a high level of organization for the reliable identification of the carcasses in large chillers. On the other hand, stress monitoring and immediate action in case of elevated stress levels (e.g. by optimizing lairage conditions related to showering, group size, and duration or by re-instructing the personnel that moves the pigs towards the stunning equipment) is not possible.

For both reasons, there is a need for stress-related variables that can be used for stress assessment in research and stress monitoring in practice. In the following, mainly the variables that have been used to assess the stress response in this thesis will be discussed. Furthermore, suggestions will be made how to monitor stress in commercial processing plants.

Stress assessment in research

Stress can be defined as 'loss of control' (Weiss, 1970) or 'a state of threatened homeostasis' (Clark, 1997) and is accompanied by behavioral and physiological changes. Behavioral changes can be easily observed but are labor-intensive, difficult to quantify, and not feasible in large groups of pigs. The time that elapses until 'normal' behavior is resumed can be a useful measure of stress (Broom and Johnson, 1993) and may be used to assess, for example, the stressor level of mixing unfamiliar pigs or transport. However, in many cases pigs are not given the opportunity nor the time to resume 'normal' behavior which is valid for most stressors that may occur during the last minutes to hours before slaughter. In these cases, objective measurement of physiological and biochemical changes in response to stressors appears more appropriate.

Catecholamines. Though catecholamines indicate reliably and immediately a stressful event, the high reactivity to the blood sampling procedure in the live animal, the fast clearance rate, as well as their high susceptibility to degradation once sampled make them very demanding. Moreover, in experiments involving stunning, be it electrical, CO_2, or mechanical, catecholamines cannot be used anymore as stress indicators because stunning exerts a larger effect than the stressor itself (Chapter 4; Althen et al., 1977; Troeger, 1989). Measurement of catecholamines in urine, the main elimination route, possibly offers an alternative because levels measured in urine represent the accumulation of the last hours (Hay et al., 2000) and are therefore not likely to be affected by stunning. However, they might inadequately reflect events that occurred during the last minutes preslaughter.

Glucocorticoids. Cortisol, the glucocorticoid commonly measured in pigs, is frequently measured in experiments assessing the effects of transport and lairage on animal welfare. Cortisol may be elevated in response to stress within few minutes but needs about 10 to 30 min to reach peak concentrations (Chapter 4, 5; Becker et al., 1985; Jensen-Waern and Nyberg, 1993). Generally, glucocorticoids increase more slowly than

catecholamines, persist longer, and are more stable during and after sampling. Moreover, cortisol can also be measured in saliva and although values differ from plasma cortisol, this method is valuable in non-invasive stress assessment. However, the use of cortisol in stress assessment is associated with other difficulties that have to be taken into account because they may influence or compromise altogether the meaningfulness of cortisol measurements. These difficulties are mainly related to

- differences in basal cortisol levels, which may be influenced by genetics, sex, circadian rhythm, and coping characteristics (Geers et al., 1994; Ruis et al., 1997, 2001),
- differences in adrenal responsiveness, i.e. the same stressor may increase cortisol to a different extent in individual pigs depending on their adrenal responsiveness (Hennessy et al., 1986; Borell and Ladewig, 1992),
- blunted cortisol response after repeated exposure to stressors due to feedback mechanisms (River and Vale, 1983),
- differences in speed of recovery after exposure to a stressor, depending on the type of stressor and the 'recovery profile' of an animal (García and Armario, 2001; Márquez et al., 2002),
- disability to differentiate between high-intensity stressors due to a 'ceiling effect' at intermediate ACTH (adrenocorticotrope hormone) levels so that circulating glucocorticoids no longer reflect ACTH release (Hennessy and Levine, 1978; Kant et al., 1983).

In concert with the aforementioned aspects, the last point might possibly be one reason for the poor correlations between cortisol and pork quality (Chapter 4; Shaw et al., 1995; Warriss et al., 1998), presuming that there is no similar ceiling effect for the magnitude of stress and its effect on pork quality. Taken together, cortisol certainly is valuable in the assessment of stress and animal welfare, not in the last place due to its relatively inexpensive analysis, but its limitations have to be taken into account.

Blood lactate. Lactate is formed in muscles as a result of anaerobic glycolysis, which starts at approximately 50 to 70% of the maximal O_2 uptake, i.e. well below the maximum capacity (Katz and Stahlin, 1988). As such, lactate is a measure of physical and not of psychological stress. In agreement, Maas (1976; cited by Steffens, 1999) mixed unfamiliar pigs and prevented fighting by a fence as a form of psychological stress without a physical component and did not observe an increase in blood lactate. Fixation by a snare restraint, on the other hand, resulted in increased blood lactate levels probably due to the physical resistance to the confinement, accompanied by an increased muscular tension (Maas, 1976; cited by Steffens, 1999; Neubert et al., 1996). Moving the pigs to the stunning area can be both physical and psychological highly stressful, resulting in both high lactate and high cortisol levels (Chapter 4-5; Warriss et al., 1994; Hartung et al., 1997). The excitability of the pigs, the design of the races, the slaughter line speed, and the handling by the stock personnel are important

determinants. Moving too large groups as well as factors such as reflection and air movement (Grandin, 1996) may cause the pigs to turn around and provoke a 'flight' reaction which may eventually result in a similar physical exercise level as the high stressor treatment in Chapter 3-6. One might assume that blood lactate is a good indicator for stress level and subsequent pork quality above all when such extreme stressor levels as tested in this thesis are compared with each other. On the contrary, blood lactate showed also within the minimal stressor treatment a high correlation with drip loss. This correlation was even stronger within the minimal than within the high stressor group ($r = 0.65$ vs. 0.38, respectively). Although suboptimal transport and lairage conditions increased the pig's response to the high stressor treatment, which was reflected in elevated blood lactate levels, the various transport and lairage treatments on its own did not affect blood lactate (Chapter 5). This might be related to the fact that either the physical exercise level was too low or the lactate that might have been formed in response to, for example, the long and rough transport treatment was already metabolized during subsequent lairage and could not be detected anymore at exsanguination. Accordingly, Jensen-Waern and Nyberg (1993) found blood lactate to return to pre-exercise values within 60 min. Blood lactate is thus an excellent, inexpensive and reliable, indicator for the stressor level immediately preslaughter because in this period the magnitude of stress is in most cases correlated with physical activity. Earlier events, where the psychological nature of the stressor prevails, might increase blood lactate not sufficiently and persistently enough to serve as a stress indicator.

Other. Unlike blood lactate and cortisol, most other stress indicators have not been related to pork quality but were mainly evaluated regarding their meaningfulness for animal welfare assessment. Creatine phosphokinase (CPK) is related to physical stress and was shown to be increased in slaughter systems that were considered as stressful (Weeding et al., 1993; Warriss et al., 1994) and that produced pork of an inferior quality (Warriss et al., 1994). Vasopressin can be used for the assessment of travel sickness (e.g. Bradshaw et al., 1996). Beta-endorphin, having the same precursor as ACTH, was reported to be not a reliable indicator for welfare during transport (Bradshaw et al., 1996). Perremans (1999) suggested that beta-endorphin can be used mainly for the detection of relatively short-term acute stressors. When working with few experimental pigs and under very controlled conditions, the continuous registration of body temperature, heart rate, and heart rate variability, which are affected during a stress response, can be useful (Geers et al., 1994; Perremans et al., 1998; reviewed by Ruis, 2001). However, the separation between stress, physical exercise, and environmental influences such as outside temperature have to be considered in the interpretation of results.

In summary, there is not one single stress indicator that is able to deliver complete and reliable information for a broad range of stressors. A combination of several variables will prove most useful and, where possible, visual observations should be used to

support physiological measurements. The selection will be largely determined by the nature of the stressor, the method of sampling (invasive, non-invasive), and the number of pigs in the experiment.

Stress monitoring in practice

Blood lactate. Chemical analysis of stress variables are costly and time consuming which makes them unsuitable for stress monitoring in practice. In sports physiology, there are portable blood lactate analysers that measure lactate quickly and inexpensively. In humans, the accuracy appears to be acceptable (e.g. Pinnington and Dawson, 2001) but from 10 mmol/L onwards, blood lactate concentrations are underestimated; 0.8 - 20 mmol/L seems to be the maximum range for these blood lactate analysers (Evans and Golland, 1996). In Chapter 4, even within the minimal stressor group the range of blood lactate that was measured, with values varying from 3.2 to 28.2 mmol/L, would exceed the analytical range of the aforementioned devices.

pH. A frequently measured variable, which is closely related to blood lactate and has a certain predictive value for pork quality (Chapter 2, 4), is muscle pH. The measurement of pH in blood at exsanguination has proven to be technically difficult due to the blood clotting around the pH electrode and in addition, blood pH may be affected by the method of stunning (Chapter 4). Muscle pH measured around 30 to 45 min postmortem has been traditionally used to monitor and predict pork quality. In addition to the disadvantages mentioned in Chapter 4, namely the possible influences of preceding (carcass) handling factors such as shackling and scalding, this measurement is also time and labour intensive and, with high line speeds, little accurate. Automation of the pH measurement seems hardly possible and consequently this method will probably remain a 'spot-check' monitoring tool.

Temperature. Stress-enhanced metabolism may result, promoted by the poor ability of the pig to dissipate heat, in an increased body and carcass temperature (Chapter 3-5; Brown et al., 1998; van der Wal et al., 1999). Although correlations with pork quality were rather low (Chapter 2 and 4), elevated muscle temperatures were nevertheless associated with both an increased stressor level and an inferior pork quality (Chapter 2 and 4). A temperature probe is far less awkward in handling than a pH electrode, but the invasive measurement of muscle temperature at 30-45 min postmortem still faces similar difficulties as the pH measurement regarding preceding carcass handling, line speeds, and automation. However, temperature can also be measured non-invasively by infrared sensitive sensors. Gariepy et al. (1989) screened with infrared directly before stunning 2000 pigs at the dorsal neck region and classified them into four temperature classes. Only 49 pigs were allocated into the highest temperature class (> 32°C). Between randomly selected pigs from the lower temperature classes, there were no differences in the incidence of PSE or DFD meat. However, for the highest temperature class, a decrease from 50 to 29% in 'normal' pork was observed. On the basis of these results, Milligan et al. (1998) in a study involving various ante- and

postmortem treatments also measured skin temperature. Infrared measured temperature did not show a discernable pattern regarding the visually determined PSE incidence. On the other hand, pork quality was best for the group of pigs that spent 2 h in lairage and worst for the pigs that received zero lairage. Infra-red temperature was clearly different for these lairage groups, with 33.2, 30.4, 29.3 and 28.1°C for 0, 1, 2, and 3 h of lairage, respectively. Although infrared thermography is probably not able to assess animal welfare or predict pork quality on an individual basis, it might offer the possibility to identify risk groups that exhibit an elevated body temperature, possibly as a result of inadequate preslaughter handling. The use of infrared sensitive cameras that are placed shortly before or after stunning may help to automate the measurement. A major challenge remains the measurement site because the skin temperature may be easily influenced by environmental conditions and does not necessarily reflect deep body (muscle) temperature (Zinn et al., 1985; Wendt et al., 1997). Showering during lairage, for example, may reduce the skin temperature for a rather long period. A similar effect is provoked by contact with cold surfaces. In Figure 3, the effects of a disturbed blood circulation, caused by a ear tag (left picture, left ear to the observer) are shown, as well as the effect of leaning against the (cold) pen wall (dark spot on the left hind leg).

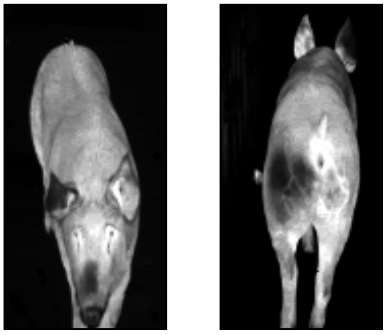


Figure 3. Surface temperature measurement by an infrared sensitive camera. Dark spots indicate a lower temperature than light spots (reproduced with the friendly permission of B. B. Houx, Utrecht University, The Netherlands, Department of Ethology and Animal Welfare).

Sound. Pigs may express acute distress or discomfort by vocalization. White et al. (1995) studied the use of vocalization measurement to assess the distress during castration of piglets and found not only an increased heart rate but also a higher frequency of highest energy measurements of vocalization for piglets castrated without a local anesthetic compared with piglets that were locally anesthetized. Under commercial slaughter conditions, however, environmental noise levels are high. Warriss et al. (1994) observed that the processing plants, which were subjectively scored as 'high stress' and were associated with high blood lactate values, produced also the highest sound levels measured directly before stunning as well as the worst pork quality. It is not clear whether sound measurements can also be used to assess variation in the stress

level within a processing plant or whether only relatively large differences in stress levels between various plants can be detected.

In summary, there are no readily available methods to monitor on a continuous basis the stress level of slaughter pigs. Developing the above listed possibilities for application in practice will be time-consuming and costly and only large operations will be able to afford it. Spot-checking more labor-intensive variables such as 30-45 min pH or manually measured blood or muscle temperature may represent an alternative although this will lead to long-term assessment of handling quality rather than provide an information tool for immediate action.

Muscle energy - strategies to restrict muscle glycogen content

Results presented in Chapter 4, which were confirmed in the study reported in Chapter 5 (results not shown), suggested that strategies to limit the amount of muscle glycogen can to some extent restrict the damage to pork quality that is caused by high preslaughter stress levels. However, glycolytic potentials, an approximation of the amount of glycogen present in the muscle in vivo, varied greatly not only between but also within a preslaughter stressor treatment as well as within a slaughter group (Chapter 4 and 5). Fernandez and Tornberg (1991) reviewed factors that may influence muscle glycogen and ultimate pH in pigs. It seems that the processing plants have very few possibilities to restrict muscle glycogen without compromising animal welfare and risking energy depletion. In the following, independent from whether or not the processing plant has control, some possible reasons for the variation as well as strategies to restrict muscle glycogen are discussed.

If zero or very short durations are disregarded, increasing feed withdrawal time alone beyond the normally recommended 12 to 18 h seems to have limited effects. Only in combination with physical activity such as fighting during lairage, prolonged feed withdrawal results in decreased glycogen levels or increased final pH values (Fernandez and Tornberg, 1991; Bidner et al., 1999a,b).

Effects of transport and lairage on muscle glycogen are very variable and depend also on other factors such as for example environmental temperatures and mixing of unfamiliar pigs (Fernandez and Tornberg, 1991). In Chapter 5, it was studied whether transport and lairage conditions within the range that was encountered in the study reported in Chapter 4 were responsible for the differences in glycolytic potential that were observed in that study. Effects of the transport and lairage treatments were very small (Chapter 5). Consequently, it must have been other factors that caused the variation in muscle glycogen content reported in Chapter 4 and which was again observed in the study reported in Chapter 5.

Ciobanu et al. (2001) and Fields et al. (2002) reported that genes distinct from the well-known RN$^-$ gene influence muscle glycogen and also the ultimate pH of pork. Possibly, genetic differences within a pig line may determine, at least partly, muscle glycogen content of an animal and cause variation independent from preslaughter handling.

Moreover, selection for decreased muscle glycogen is possible (Le Roy et al., 1998) and may offer a possibility to improve pork quality without compromising animal welfare.

The feed composition was not different for pigs within a slaughter group (Chapter 4) and the same for all pigs in Chapter 5. Nevertheless, diet composition may represent a tool to reduce muscle glycogen in an animal friendly way. Rosenvold et al. (2001a,b,2003) and Rosenvold and Andersen (2003) showed that a diet low in digestible carbohydrates and high in fat has potential to reduce muscle glycogen and to improve pork quality. Leheska et al. (2002) studied a diet low in digestible carbohydrates and high in protein and did not find a similar effect. From human sports physiology, it is known that a high pre-exercise carbohydrate intake in combination with a specific training schedule, a so-called 'carbohydrate loading', may increase muscle glycogen levels (reviewed by van Loon, 2001; H. Kuijpers, personal communication). When preceded by a diet rich in fat, however, subsequent carbohydrate utilization during exercise seems to be impaired (reviewed by Kiens, 2001). Rosenvold et al. (2001) found an improved pork quality but no differences in final pH by the strategic feeding. In the same study, a higher 45-min pH indicated a slower postmortem metabolism for the strategically-fed pigs. Thus effects on pork quality may actually be due to the high fat content that slowed (ante- and postmortem) carbohydrate utilization.

Behavioural differences between individual pigs, e.g. physical activity during transport and lairage, which have not been assessed in the studies presented in this thesis, may also play a role. In support, Fernandez et al. (1994) showed that glycogen content in the semispinalis muscle was closely correlated to aggressive behaviour of the pigs. Preslaughter management that equally promotes resting behaviour for all pigs and avoids agitation cannot restrict but at least help to reduce the large variation in muscle glycogen. Reducing the mixing of unfamiliar pigs, avoiding prolonged standstills during transport and before unloading at the processing plants (which promote fighting and an increase in temperature due to failure of ventilation etc.), and optimal group sizes in lairage are only some examples that may prevent the latent differences in physical and psychological coping capacity between individual pigs to become evident.

This exposition makes by no means claim to be exhaustive. Nevertheless it can be stated that there is still a gap in knowledge about the (quantitative) effects of genetic, nutrition, handling and other, unknown, factors that control muscle glycogen. Moreover, results reported in Chapter 6 and its Appendix imply that more muscles than only the longissimus have to be considered. When strategies to restrict muscle glycogen are developed, care has to be taken to avoid negative effects in more oxidative, DFD prone muscles, where restriction of muscle glycogen may increase or even raise a quality problem rather than solving it.

Concluding remarks

Results of this thesis have shown that stress in the immediate preslaughter period deteriorates pork quality and increases the variation in both drip loss and pork color.

Suboptimal transport and lairage conditions as well as high muscle glycogen levels alone did not affect pork quality; however, they aggravated the negative effects of high stress immediately before slaughter. On the other hand, the method of stunning and the rate of chilling exerted, independent from stressor level, only a limited and far smaller effect on the color and the water-holding capacity of pork compared with preslaughter stress. Therefore, the first measures to improve pork quality should aim at avoiding or reducing stress in the immediate preslaughter period, a process in which stress monitoring may represent an important tool.

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Summary
Samenvatting
Zusammenfassung
Resumen

Summary

Color and water-holding capacity are two important pork quality aspects. At the moment of purchase, color is crucial in quality assessment. Retention, gains, and losses of water, on the other hand, affect the weight and thus the economical value of the meat. There exists a large variation in both attributes which gives rise to complaints by both producers and consumers. This thesis deals with the effects of transport, lairage and stress level immediately before slaughter, the stunning method used, and the rate of carcass chilling. Particular attention was paid to the interaction between the various pre- and postslaughter handling factors. The intention was not only to improve the average level but also to reduce the variation of the water-holding capacity and the color of pork.

To guarantee the direct applicability of the results, all studies were conducted in commercial processing plants. The three plants, where the experiments were carried out, differed in layout, stunning system (electrical vs. CO_2), and rate of chilling. All pigs slaughtered within these experiments were end-products free of the halothane gene. In all studies, final pork quality attributes, predominantly related to color and water-holding capacity, were measured in the loin. In Chapter 6 and the Appendix to Chapter 6, quality was also assessed in muscles of the shoulder and the ham. Additionally, blood-based stress indicators were measured at exsanguination in the studies reported in Chapter 4 and 5. Muscle glycolytic potential, an approximation of the in vivo muscle glycogen content, was measured in the studies reported in Chapter 4, 5, and 6.

In the first study, three different processing plants were compared regarding pork quality (**Chapter 2**). The plants differed in the layout of the races, stunning method (electrical vs. CO_2), and chilling systems (conventional vs. rapid). Various groups of slaughter pigs were each divided into three and sent on consecutive days to the three plants. Factors that may influence pork quality but were not directly associated with the processing plants were standardized. Within the same group, farm (and genetic) origin, feed withdrawal, as well as transport and lairage duration were kept constant. Both plants employing electrical stunning produced pork with a slightly better water-holding capacity compared with the plant using CO_2 stunning. In addition, the plant employing both electrical stunning and rapid chilling produced pork with the lowest (best) electrical conductivity values, one of the attributes measured to describe the water-holding capacity.

In a follow-up study, at one of the above plants it was investigated whether increasing the rate of chilling could compensate for the negative effects on pork quality that high stressor levels in the immediate preslaughter period may have (**Chapter 3**). Pigs were subjected to either a minimal or a high stressor treatment during the last five minutes before slaughter. Later on, carcasses underwent either conventional chilling at 4°C for 22 h or rapid chilling by first passing through a three-phase chilling tunnel (-15, -10,

and -1°C for 15, 38, and 38 min, respectively), followed by storage at 4°C until 22 h postmortem. Rapid chilling, as opposed to conventional chilling, improved electrical conductivity in the loin regardless of preslaughter stressor level but could not compensate for the detrimental effects of stress. Except for an increased internal reflection in response to rapid chilling, pork color and other attributes related to water-holding capacity were not affected by the chilling system. It was concluded that increasing chilling rate is not a suitable method to resolve pork quality problems caused by inadequate preslaughter handling.

The same stressor treatments were used in the study reported in **Chapter 4**. At two plants, employing either electrical or CO_2 stunning, physiological changes elicited in response to stress in the immediate preslaughter period were studied and linked to pork quality characteristics. At both plants, the high stressor treatment resulted in inferior pork quality attributes. The effect was greater on water-holding capacity than on pork color, with drip losses increased by 56%. In contrast to the study reported in Chapter 2, the plant employing CO_2 stunning produced pork with a slightly better water-holding capacity compared with the plant employing electrical stunning. Of all stress indicators measured at exsanguination, only blood lactate was strongly correlated with pork quality attributes. Regression analyses revealed that blood lactate and glycolytic potential accounted for 52 and 48% of the variation in drip loss and L* value, respectively. In combination with high preslaughter stress, high glycolytic potentials were related to increased drip losses. It was concluded that high preslaughter stress impairs pork quality, with high muscle energy levels aggravating the negative effects of preslaughter stress.

In the next experiment (**Chapter 5**), it was investigated whether the large variation in glycolytic potentials, which was found in the previous study, was due to differences in transport and lairage. Moreover, it was studied whether the negative effects of high stress immediately preslaughter were affected by preceding handling. Pigs were assigned to either short and smooth or long and rough transport (50 min or 3 h, respectively), long or short lairage (3 h or <45 min, respectively), and minimal or high preslaughter stress. The effects of the transport and lairage treatments on muscle glycolytic potential and subsequent pork quality were minor. It was concluded that within these ranges transport and lairage treatments were probably not responsible for the large variation in muscle glycolytic potential. Regarding water-holding capacity, on the other hand, comparisons with the "optimal" handling (short and smooth transport, long lairage, minimal stress) revealed that the negative effects of high stress were aggravated by suboptimal transport and lairage conditions. High stress alone increased electrical conductivity by 56% whereas high stress in combination with short lairage led to an 88%-increase. However, stress in the immediate preslaughter period contributed most and was the major responsible factor for an impaired pork quality.

In **Chapter 6**, it was investigated whether the effects of the same transport, lairage, and stressor treatments depended on the fibre type composition of the muscle. Muscle

glycolytic potential and pork quality were assessed in the predominantly glycolytic longissimus muscle (loin) as well as the more oxidative supraspinatus (shoulder) or serratus ventralis (Boston Butt) muscles, respectively. The effects of transport and lairage conditions were similar in both muscle types. For the high preslaughter stressor treatment, on the other hand, several interactions between stressor level and muscle type were observed. Effects of stressor levels on water-holding capacity were larger in the glycolytic longissimus muscle, whereas effects on color were more prominent in the oxidative serratus ventralis muscle. It was concluded that intense physical activity and/or high psychological stress levels preslaughter promote the development of the PSE condition (pale, soft, exudative meat) in glycolytic muscles while at the same time increasing the tendency towards DFD (dark, firm, dry meat) in oxidative muscles.

In the **Appendix to Chapter 6**, the effects of the transport, lairage, and stressor treatments are described exclusively with regards to the color in various pork muscles (longissimus lumborum, longissimus thoracic, and semimembranosus muscles (all predominantly glycolytic muscle types), and the more oxidative serratus ventralis muscle). The effects of the various preslaughter handling treatments were different depending on the muscle studied, basically confirming results reported in Chapter 6. Moreover, there existed considerable variation within the loin; the cranial part (longissimus thoracic) was more sensitive to high preslaughter stressor levels than the caudal part (longissimus lumborum). It is recommended to assess the effects of preslaughter handling on pork quality not only in one but in several muscles.

In the **General discussion**, results of the previous studies were summarized regarding the relative importance of the individual pre- and postslaughter factors for pork color and water-holding capacity as well as their effect on the variation in the two attributes. Additionally, methods to assess stress under practice and research conditions were discussed as well as some strategies to control muscle glycogen content.

From results presented in this thesis it is concluded that:

- Stress in the immediate preslaughter period deteriorates the level and increases the variation of pork quality. Of all factors studied, stressor level emerged as the most important one.
- Increasing the rate of chilling cannot compensate for the negative effects of high stress.
- The effects of stunning method on pork color and water-holding capacity were, compared with stressor level, minor and not consistent over studies.
- Transport and lairage conditions, within the ranges studied in this thesis, have little effect on pork quality. In combination with high stress prior to slaughter, however, suboptimal transport and lairage conditions may aggravate the effects of high stress in the immediate preslaughter period.

- High levels of muscle glycogen at the moment of slaughter alone do not affect the color and the water-holding capacity of pork. In combination with high stress, however, high levels of muscle glycogen may further impair pork quality.

Samenvatting

Kleur en waterhoudendvermogen zijn twee belangrijke kwaliteitskenmerken van varkensvlees. Terwijl de kleur een cruciale rol speelt in de aankoopbeslissing, beïnvloeden toe- en afnames in het vochtgehalte het gewicht en daarmee de financiële waarde van het vlees. Er bestaat een grote variatie in beide kenmerken wat aanleiding geeft tot klachten van zowel producenten als ook consumenten. Het voorliggende proefschrift gaat over de effecten van het transport naar de slachterij, de wachttijd op de slachterij en het stressniveau direct voor het slachten, de verdovingsmethode en de koelsnelheid. Speciale aandacht werd geschonken aan de interacties tussen de verschillende factoren. Het doel was niet alleen de verbetering van het gemiddelde niveau van vleeskwaliteit maar ook het verminderen van de variatie in de kleur en het waterhoudendvermogen van varkensvlees.

Om de directe toepasbaarheid van de onderzoeksresultaten te garanderen, zijn alle studies binnen commerciële slachterijen uitgevoerd. De drie bedrijven waar de experimenten plaats hebben gevonden, verschilden in opzet, verdovingsmethode en koelsnelheid. Alle varkens die binnen deze experimenten werden gevolgd, waren vrij van het halothaangen. In alle studies werd vleeskwaliteit in de karbonadespier gemeten. Dit betrof met name kenmerken gerelateerd aan de kleur en het waterhoudendvermogen. In Hoofdstuk 6 en in de Appendix van Hoofdstuk 6 werd de vleeskwaliteit ook in schouder- en hamspieren gemeten. Aanvullend zijn in Hoofdstuk 4 en 5 stressindicatoren in het steekbloed gemeten. Het glycolytisch potentieel, een schatting van het in vivo glycogeengehalte in spieren, is in Hoofdstuk 4, 5 en 6 gemeten.

In de eerste studie zijn drie verschillende slachterijen op vleeskwaliteitsaspecten met elkaar vergeleken (**Hoofdstuk 2**). De slachterijen verschilden in opzet van de wachtruimte en de drijfgangen, verdovingsmethode (electrisch vs. CO_2) en de koelsnelheid (conventioneel vs. snelkoeling). Groepen vleesvarkens werden telkens in drie opgedeeld en op achtereenvolgende dagen naar de drie slachterijen gebracht. Factoren die invloed kunnen hebben op de vleeskwaliteit maar niet direct aan de slachterijen gerelateerd waren, werden constant gehouden. Binnen dezelfde groep waren er geen verschillen in bedrijfs- (genetische) achtergrond, voeronthouding of transport en wachttijden. De twee slachterijen met een electrisch verdovingssysteem produceerden vlees met een beter waterhoudendvermogen vergeleken met de slachterij waar de varkens door CO_2 werden verdoofd. Bovendien was de electrische geleidbaarheid, een kenmerk gerelateerd aan het waterhoudendvermogen, het laagst (oftewel het best) in het vlees wat in de slachterij werd geproduceerd die zowel electrische verdoving als ook een snelkoeling toepaste.

In een vervolgstudie werd in één van de in Hoofstuk 2 bestudeerde slachterijen onderzocht of het verhogen van de koelsnelheid de negatieve effecten van een hoog

stressniveau onmiddelijk voor het slachten op de vleeskwaliteit kon compenseren (**Hoofdstuk 3**). Gedurende de laatste vijf minuten voor het slachten ondergingen de varkens een behandeling die of met weinig of met veel stress gepaard ging. Daarna werden de karkassen of conventioneel bij 4°C tot 22 uur postmortem gekoeld of passeerden eerst een 3-fases snelkoeltunnel (-15, -10 en -1°C gedurende respectievelijk 15, 38 and 38 min) alvorens de conventionele koelfase bij 4°C tot 22 uur postmortem. Een verhogde koelsnelheid verbeterde de electrische geleidbaarheid in de karbonadespier onafhankelijk van het stressniveau voor het slachten. De negatieve effecten van stress konden echter niet gecompenseerd worden. Behalve een verhoogde interne reflectiewaarde voor de snelgekoelde karkassen waren er verder geen effecten van de koelsnelheid op de kleur en het waterhoudendvermogen van het vlees. Concluderend kan worden gesteld dat een verhoging van de koelsnelheid geen geschikt middel is om kwaliteitsproblemen op te lossen die zijn veroorzaakt door ruw om te gaan met varkens voor het slachten.

In **Hoofdstuk 4** zijn dezelfde stressbehandelingen ingezet als in Hoofdstuk 3. Op twee slachterijen waar de varkens of electrisch of met CO_2 werden verdoofd, zijn de fysiologische veranderingen in bloed en spier als reactie op de verschillende stressniveaus voor het slachten bestudeerd. Zowel na electrisch als ook na CO_2 verdoving veroozaakte stress direct voor het slachten een verslechtering van de vleeskwaliteit. Het effect was groter op waterhoudendvermogen dan op kleur van het vlees. De drip verliezen waren met 56% verhoogd. In tegenstelling tot de resultaten van Hoofdstuk 2 produceerde de slachterij met een CO_2 verdovingssysteem vlees met een iets beter waterhoudendvermogen vergeleken met de slachterij die de varkens electrisch verdoofde. Van alle stressindicatoren in het bloed gemeten, toonde alleen bloedlactaat een goede correlatie met vleeskwaliteit. Bloedlactaat en het glycolytisch potentieel verklaarden gezamenlijk respectievelijk 52 en 48% van de variatie in dripverliezen en de L* waarde (lichtheid) van het vlees. Een hoog glycolytisch potentieel was alleen in combinatie met een hoog stressniveau direct voor het slachten gerelateerd aan verhoogde dripverliezen. Conclusie was dat een hoog stressniveau direct voor het slachten tot een slechtere vleeskwaliteit leidt en dat een hoog spierenergieniveau het effect van stress kan verergeren.

In het volgende experiment (**Hoofdstuk 5**) werd onderzocht of de grote variatie in het glycolytische potentiëel die werd aangetroffen in de vorige studie, was veroorzaakt door verschillen in transport en wachttijden. Bovendien is bestudeerd of de negatieve effecten van veel stress voor het slachten werden beïnvloedt door voorafgaande gebeurtenissen. De varkens werden ingedeeld in groepen die verschilden in transportomstandigheden (korte en soepel (50 min) of lang en ruig (3 uur)), wachttijd (lang (3 uur) of kort (<45 min)) en stress niveau (minimaal of hoog). De effecten van de transport- en wachttijdbehandelingen op het glycolytisch potentieel en de vleeskwaliteit waren gering. Dit leidde tot de conclusie dat binnen de onderzochte range de transport- en wachttijdomstandigheden waarschijnlijk niet verantwoordelijk

waren voor de grote variatie in glycolytisch potentieel. Vergelijkingen met de 'optimale' behandeling (korte, soepele transport, lange wachttijd, weinig stress) openbaarden echter dat met betrekking tot het waterhoudendvermogen de negative effecten van stress verergerd werden door suboptimale transport en wachttijdomstandigheden. Een hoog stressniveau op zich zelf verhoogde de electrische geleidbaarheid met 56% terwijl veel stress in combinatie met een (te) korte wachttijd tot een toename van 88% leidde. Maar het stressniveau direct voor het slachten speelde de belangrijkste rol in het ontstaan van vleeskwaliteitsproblemen.

In **Hoofdstuk 6** is onderzocht of de effecten van dezelfde transport-, wachttijd- en stressbehandelingen als in Hoofdstuk 5 afhankelijk waren van de spiervezel-samenstelling. Het glycolytisch potentieel en de vleeskwaliteit werden gemeten in de voornamelijk glycolytische longissimus (karbonadespier) en in de meer oxidatieve supraspinatus (schouder) of serratus ventralis (neck) spier. De effecten van de verschillende transport- and wachttijden waren in beide spiertypen vergelijkbaar. Daarentegen waren er interacties tussen de spiertypen en de effecten van stress. Het negatieve effect van een hoog stressniveau direct voor het slachten op het waterhoudendvermogen was groter in de glycolytische karbonadespier, terwijl de effecten van stress op de vleeskleur duidelijker te zien waren in de oxidatieve serratus ventralis spier. Dit leidde tot de conclusie dat intensieve fysieke inspanning en/of psychische stress in glycolytische spieren het ontstaan van PSE (bleek, zacht en waterloslatend) vlees bevordert terwijl dezelfde omstandigheden in oxidatieve spieren DFD (donker, vast en droog) vlees kunnen veroorzaken.

In de **Appendix van Hoofdstuk 6** zijn de effecten van de transport, wachttijd en stressor behandelingen beschreven met betrekking tot de kleur van verschillende spieren (longissimus lumborum, longissimus thoracic en semimembranosus spier (allen overwegen glycolytische spiertypes), en de meer oxidatieve serratus ventralis spier). In overeenstemming met de uitkomsten beschreven in Hoofdstuk 6 waren de effecten van de verschillende behandelingen afhankelijk van de spier. Bovendien was er een behoorlijke variatie binnen de karbonadespier; het craniale gedeelte (longissimus thoracic) was gevoeliger voor hoge stressniveaus dan het caudale gedeelte (longissimus lumborum). Het wordt aanbevolen om de effecten van behandelingen in de fase voor het slachten niet alleen in één maar in meerdere spieren te bestuderen.

In de **Algemene discussie** zijn de resultaten van de verschillende studies samengevat wat betreft de betekenis van de individuele factoren voor de kleur en het vochvasthoudend vermogen van varkensvlees alsmede hun effect op de variatie van deze twee kwaliteitskenmerken. Bovendien zijn er verschillende mogelijkheden bediscussieerd hoe stress onder praktijk- of onderzoeksomstandigheden kan worden gemeten. Ook werd nagegaan hoe het glycogeengehalte van spieren kan worden gecontrolleerd.

De in dit proefschrift gepresenteerde resultaten leidden tot de volgende conclusies:

- Stress onmiddelijk voor het slachten verslechtert het niveau van vleeskwaliteit en vergroot de variatie. Van alle factoren die bestudeerd zijn, kwam het stress niveau als belangrijkste naar voren.
- Verhogen van de koelsnelheid kan niet kompenseren voor de negatieve effecten van stress.
- De effecten van de verdovingsmethode op de kleur en het waterhoudend-vermogen van vlees zijn, vergeleken met de effecten van stress, gering en niet consistent over de verschillende studies heen.
- Transportomstandigheden en wachttijden hebben, binnen de in dit proefschrift bestudeerde range, een gering effect op de uiteindelijke vleeskwaliteit. In combinatie met hoge stress niveaus direct voor het slachten kunnen suboptimale transport- en wachtomstandigheden echter de negatieve effecten van stress verergeren.
- Hoge glycogeengehaltes in spieren hebben op zichzelf geen effect op de kleur en het waterhoudendvermogen van varkensvlees. Echter in combinatie met veel stress direct voor het slachten, kunnen hoge glycogeengehaltes de negatieve effecten van stress versterken.

Zusammenfassung

Die Farbe und das Wasserbindevermögen sind zwei wichtige Qualitätsmerkmale von Schweinefleisch. Während die Fleischfarbe eine zentrale Rolle bei der Kaufentscheidung spielt, beeinflussen Veränderungen des Wassergehaltes das Gewicht und damit den finanziellen Wert des Fleisches. Beide Merkmale weisen eine große Variation auf, was sowohl von Produzenten als auch Konsumenten beanstandet wird. In der vorliegende Doktorarbeit wurden die Auswirkungen des Transports zum Schlachthof, der Ruhezeit im Wartestall auf dem Schlachthof, des Stressniveaus direkt vor der Schlachtung, der Betäubungsmethode und der Schnellheit der Schlachtkörperkühlung auf die Fleischqualität untersucht. Von besonderem Interesse waren die Wechselwirkungen zwischen den einzelnen Faktoren. Ziel war nicht allein eine Verbesserung des allgemeinen Niveaus der Fleischqualität, sondern auch die Verringerung der Variation in der Fleischfarbe und des Wasserbindevermögens von Schweinefleisch.

Um die direkte Anwendbarkeit der Resultate zu gewährleisten, wurden alle Studien in kommerziellen Schlachthöfen durchgeführt. Die drei Schlachthöfe, in denen die Experimente stattfanden, unterschieden sich in der Anlage der Warteräume und der Zutriebswege, der Betäubungsmethode und der Schlachtkörperkühlung. Alle Schweine, die im Rahmen dieser Arbeit geschlachtet wurden, waren halothan-negativ. In allen Studien wurde die Fleischqualität im Kotelettmuskel gemessen. Vor allem Merkmale, die mit der Fleischfarbe und dem Wasserbindevermögen verbunden sind, wurden erfasst. In Kapitel 6 und im Appendix zu Kapitel 6 wurde die Fleischqualität auch in Muskeln der Schulter und des Schinkens gemessen. Zusätzlich wurden in den Kapiteln 4 und 5 Stressindikatoren im Stichblut analysiert. Das glykolytische Potenzial, eine Schätzung des in-vivo Glycogengehaltes im Muskel, wurde in den Kapiteln 4, 5 und 6 gemessen.

Im ersten Experiment wurden drei Schlachthöfe bezüglich der von ihnen produzierten Fleischqualität miteinander verglichen (**Kapitel 2**). Die Schlachthöfe unterschieden sich in der Anlage der Warteräume und der Zutriebswege, der Betäubungsmethode (elektrisch vs. CO_2) und der Schlachtkörperkühlung (konventionell vs. Schnellkühlung). Mehrere Gruppen Schlachtschweine wurden jeweils in drei Subgruppen eingeteilt und an aufeinanderfolgenden Tagen zu den drei Schlachthöfen transportiert. Faktoren, die die Fleischqualität hätten beeinflussen können, aber keinen direkten Zusammenhang mit den Schlachthöfen hatten, wurden konstant gehalten. Innerhalb derselben Gruppe waren der Herkunftsbetrieb (das heisst auch die Genetik), die Nüchterzeit sowie der Transport und die Ruhezeit auf dem Schlachthof identisch. Die beiden Schlachthöfe mit einem elektrischen Betäubungssystem produzierten Fleisch mit einem besseren Wasserbindevermögen, verglichen mit dem Schlachthof, der die Schweine mit CO_2 betäubte. Darüber hinaus war die elektrische Leitfähigkeit, ein Merkmal, das eng mit dem Wasserbindevermögen verbunden ist, am niedrigsten

(besten) in dem Schlachthof, der neben einem elektrischen Betäubungssystem zusätzlich eine Schnellkühlung einsetzte.

In der nächsten, auf den in Kapitel 2 beschriebenen Resultaten aufbauenden Studie (**Kapitel 3**) wurde in einem der Schlachthöfe untersucht, ob ein Erhöhen der Kühlgeschwindigkeit die negativen Effekte eines hohen Stressniveaus direkt vor der Schlachtung auf die Fleischqualität kompensieren kann. Während der letzten fünf Minuten vor der Schlachtung wurden die Schweine einer Behandlung unterzogen, die mit einem geringen oder einem hohen Stressniveau einherging. Danach wurden die Schlachtkörper entweder konventionell bei 4°C bis 22 Stunden postmortem gekühlt oder passierten erst einen 3-phasigen Schnellkühltunnel (-15, -10 und -1°C während respektive 15, 38 und 38 min) vor der konventionellen Kühlfase bei 4°C bis 22 Stunden postmortem. Eine erhöhte Kühlgeschwindigkeit verbesserte die elektrische Leitfähigkeit unabhängig vom Stressniveau vor dem Schlachten. Die negativen Effekte eines hohen Stressniveaus konnten jedoch nicht kompensiert werden. Außer einem erhöhten internen Reflektionswert für die schnellgekühlten Schlachtkörper waren weiter keine Effekte der Kühlschnellheit auf die Fleischfarbe oder das Wasserbindevermögen festzustellen. Schlussfolgernd ist eine Erhöhung der Kühlschnellheit kein geeignetes Mittel, um Qualitätsprobleme, die durch einen unangemessenen Umgang mit Schlachtschweinen entstanden sind, zu lösen.

Die gleichen Stressbehandlungen wurden in **Kapitel 4** eingesetzt. In zwei Schlachthöfen, wo entweder eine elektrische oder eine CO_2-Betäubungsanlage im Einsatz war, wurden die physiologischen Veränderungen im Blut als Reaktion auf die unterschiedlichen Stressniveaus untersucht. Sowohl nach der elektrischen als auch nach der CO_2-Betäubung verursachte ein hohes Stressniveau unmittelbar vor dem Schlachten eine Verschlechterung der Fleischqualität. Der Effekt auf das Wasserbindevermögen war größer als auf die Fleischfarbe, wobei die Tropfsaftverluste um 56% erhöht waren. Im Gegensatz zu den Resultaten aus Kapitel 2 produzierte der Schlachthof mit der CO_2-Betäubungsanlage Fleisch mit einem etwas besseren Wasserbindevermögen im Vergleich zu dem Schlachthof mit der elektrischen Betäubungsanlage. Von allen Stressindikatoren, die während des Entblutens gemessen wurden, wies lediglich der Laktatspiegel im Blut einen engen Zusammenhang mit der Fleischqualität auf. Zusammen mit dem glykolytischen Potenzial erklärte der Blutlaktatspiegel respektive 52 und 48% der Variation in den Tropfsaftverlusten und dem L*-Wert (Helligkeit) des Fleisches. In Kombination mit einem hohen Stressniveau vor dem Schlachten war ein hohes glykolytisches Potenzial mit erhöhten Tropfsaftverlusten assoziiert. Zusammenfassend kann gesagt werden, dass ein hohes Stressniveau direkt vor der Schlachtung die Fleischqualität verschlechtert und dass ein hoher Glykogengehalt im Muskel zum Zeitpunkt des Schlachtens die negativen Effekte von Stress verschlimmern kann.

Im nächsten Experiment (**Kapitel 5**) wurde untersucht, ob die große Variation des glykolytischen Potenzials, die in der vorherigen Studie festgestellt wurde, durch

Unterschiede in Transport- und Ruhezeiten verursacht wurde. Außerdem wurde untersucht, ob die negativen Effekte eines hohen Stressniveaus vor dem Schlachten durch vorherige Ereignisse beeinflusst werden. Die Schweine wurden in mehrere Versuchsgruppen eingeteilt, die sich in den Transportbedingungen (kurz (50 min) und angenehm oder lang (3 Stunden) und unangenehm), der Ruhezeit (lang (3 Stunden) oder kurz (<45 min)) und dem Stressniveau (gering oder hoch) unterschieden. Der Einfluss der Transportbedingungen und der Ruhezeit auf das glykolytische Potenzial waren gering. Sehr wahrscheinlich sind unterschiedliche Transportbedingungen und Ruhezeiten innerhalb des untersuchten Bereiches nicht für die große Variation in den glykolytischen Potenzialen verantwortlich. Vergleiche mit der 'optimalen' Behandlung (kurzer, angenehmer Transport, lange Ruhezeit und ein geringes Stressniveau) brachten aber zum Vorschein, dass, hinsichtlich des Wasserbindevermögens, die negativen Effekte eines hohen Stressniveaus direkt vor dem Schlachten durch suboptimale Transportbedingungen und Ruhezeiten verschlimmert werden. Ein hohes Stressniveau allein erhöhte die elektrische Leitfähigkeit um 56%, während in Kombination mit einer (zu) kurzen Ruhezeit die Leitfähigkeit um 88% erhöht wurde. Nichtsdestotrotz spielte das Stressniveau unmittelbar vor dem Schlachten die größte Rolle in der Entstehung von Fleischqualitätsproblemen.

In **Kapitel 6** wurde der Frage nachgegangen, ob die Effekte der gleichen Transport-, Ruhezeit- und Stressbehandlungen abhängig waren von der Muskelfaser-zusammenstellung. Das glykolytische Potenzial wurde in dem vornehmlich glykolytischen Longissimus (Kotelettmuskel) und in dem mehr oxidativen Supraspinatus oder dem Serratus ventralis (beides Muskel der Schulter) gemessen. Die Auswirkungen der verschiedenen Transport- und Ruhezeitbehandlungen waren in beiden Muskeltypen vergleichbar. Im Gegensatz dazu konnten Wechselwirkungen zwischen Muskeltyp und Stressniveau beobachtet werden. Der negative Effekt eines hohen Stressniveaus auf das Wasserbindevermögen war größer im glykolytischen Kotelettmuskel, während die Effekte auf die Fleischfarbe deutlicher im oxidativen Serratus ventralis zu sehen waren. Dies führte zur Schlussfolgerung, dass intensive körperliche Belastung und/oder psychischer Stress in glykolytischen Muskeln in PSE-Fleisch (blasses, strukturloses Fleisch mit einem geringen Wasserbindevermögen) resultiert, während in oxidativen Muskeln mehr DFD-Fleisch (dunkles, festes Fleisch mit einem (zu) hohen Wasserbindevermögen) auftritt.

Im **Appendix zu Kapitel 6** wurden die Auswirkungen der Transport-, Ruhezeit- und Stressbehandlungen auf die Fleischfarbe verschiedener Muskeln (Longissimus lumborum, Longissimus thoracis und Semimembranosus (alles vorwiegend glycolytische Muskeltypen), und der mehr oxidative Serratus ventralis) beschrieben. In Übereinstimmung mit den Resultaten in Kapitel 6 waren die Behandlungseffekte abhängig vom jeweiligen Muskel. Darüber hinaus wurde eine beachtliche Variation innerhalb des Kotelettmuskels beobachtet; der kraniale Teil (Longissimus thoracis) war empfindlicher gegenüber hohen Stressniveaus als der kaudale Teil (Longissimus

lumborum). Es wurde empfohlen, die Effekte von Behandlungen in der Phase vor dem Schlachten nicht nur in einem, sondern in mehreren Muskeln zu untersuchen.

In der **Allgemeinen Diskussion** wurden die verschiedenen Studien zusammengefasst hinsichtlich der Bedeutung der unterschiedlichen Faktoren für die Farbe und das Wasserbindevermögen von Schweinefleisch. Dabei wurden Auswirkungen auf die Variation der beiden Merkmale besonders berücksichtigt. Außerdem wurden verschiedene Möglichkeiten beurteilt, wie Stress unter sowohl Praxis- als auch experimentellen Bedingungen gemessen werden kann. Ebenfalls erörtert wurden Möglichkeiten, wie das Glykogenniveau in Muskeln beeinflusst werden kann.

Aufgrund der in dieser Arbeit vorgestellten Ergebnisse wurden folgende Schlussfolgerungen gezogen:

- Stress direkt vor dem Schlachten beeinträchtigt die Fleischqualität und vergrößert die Variation in der Fleischfarbe und dem Wasserbindevermögen. Von allen untersuchten Faktoren war das Stressniveau der wichtigste.
- Eine erhöhte Kühlgeschwindigkeit kann die negativen Effekte eines hohen Stressniveaus nicht vollständig kompensieren.
- Die Auswirkungen der Betäubungsmethode auf die Fleischfarbe und das Wasserbindevermögen waren, verglichen mit den Effekten von Stress, gering und nicht konsistent über verschiedene Studien hinweg.
- Transportbedingungen und Ruhezeiten haben, innerhalb des untersuchten Bereiches, einen geringen Effekt auf die Fleischqualität. In Verbindung mit einem hohen Stressniveau unmittelbar vor dem Schlachten können suboptimale Transport- und Ruhebedingungen jedoch die negativen Auswirkungen eines hohen Stressniveaus verschlimmern.
- Hohe Glykogengehalte in Muskeln haben für sich alleine genommen keinen Effekt auf die Farbe und das Wasserbindevermögen von Schweinefleisch. In Kombination mit einem hohen Stressniveau direkt vor dem Schlachten können jedoch hohe Glykogengehalte die negativen Effekte von Stress verstärken.

Resumen

El color y la capacidad de retención de agua son dos importantes aspectos de la calidad de la carne de cerdo. En el momento de la compra, el color juega un papel crucial en la evaluación de calidad. La retención, ganancias y pérdidas de agua, por otro lado, afectan al peso, y por consiguiente al valor económico de la carne. Existe una gran variación en ambas características, lo que da lugar a quejas tanto por parte de productores como de consumidores. Esta tesis aborda los efectos del transporte, descanso y niveles de estrés inmediatamente anteriores al sacrifico, el método de aturdido utilizado y la velocidad de refrigeración. Se ha prestado particular atención a la interacción entre los diferentes factores de manejo pre- y post-sacrificio. El objetivo no era sólo mejorar el nivel medio, sino reducir también la variación de la capacidad de retención de agua y el color de la carne de cerdo.

Para garantizar la aplicabilidad de los resultados, todos los estudios fueron llevados a cabo en plantas de procesado comerciales. Las tres plantas donde se llevaron a cabo los experimentos, diferían en diseño, sistema de aturdido, y velocidad de refrigeración. Todos los cerdos sacrificados en estos experimentos eran producto final libres del gen halotano. En todos los estudios, las características finales de calidad de la carne de cerdo, predominantemente relacionadas con color y capacidad de retención de agua, fueron medidas en el lomo. En el Capítulo 6 y en el Apéndice al Capítulo 6, la calidad de la carne fue también medida en los músculos de la paleta y el jamón. Adicionalmente, se midieron indicadores sanguíneos de estrés en el desangrado en los estudios referidos a los Capítulos 4 y 5. El potencial glicolítico muscular, una aproximación al contenido de glucógeno del músculo vivo, se midió en los estudios referidos en los Capítulos 4, 5 y 6.

En el primer estudio, tres diferentes plantas de procesado se compararon al respecto de calidad de la carne de cerdo **(Capítulo 2).** Las plantas diferían en el diseño de los pasillos, método de aturdido (eléctrico vs CO_2), y sistema de refrigeración (convencional vs rápido). Varios grupos de cerdos para sacrifico fueron divididos, a su vez, en tres grupos y enviados en días consecutivos a las tres plantas. Se estandarizaron aquellos factores de posible influencia en la calidad de la carne pero que no estaban directamente asociados a las plantas de procesado. Dentro del mismo grupo, el origen de granja (y de genética), período de ayuno así como el transporte y duración del reposo pre sacrificio, se mantuvieron constantes. Las dos plantas que utilizaban el aturdido eléctrico, produjeron carne de cerdo con una ligera mejor capacidad de retención de agua, en comparación con la planta que utilizaba el sistema de aturdido mediante CO_2. Además, la planta que combinaba el sistema de aturdido eléctrico y la refrigeración rápida producía la carne de cerdo con los más bajos (mejores) valores de conductividad eléctrica, uno de los parámetros utilizados para describir la capacidad de retención de agua.

En un estudio de seguimiento (**Capítulo 3**) a una de las plantas anteriormente mencionadas, se investigó si el incremento de la velocidad de refrigeración de las canales podría compensar los posibles efectos negativos en la calidad de la carne debido a altos niveles de estrés en el período inmediatamente previo al sacrificio**.** Los cerdos fueron sujetos a un tratamiento de mínimo o de alto nivel de estrés durante los últimos 5 minutos previos al sacrificio. Posteriormente, las canales fueron, o bien refrigeradas de forma convencional a 4ºC durante 22 horas o bien refrigeradas de forma rápida, pasando a través de un túnel de refrigeración en tres fases (-15, -10, y –1 ºC, durante 15, 38 y 38 minutos, respectivamente), seguido por un almacenaje a 4ºC hasta 22 horas post-mortem. La refrigeración rápida, frente a la refrigeración convencional, mejoró la conductividad eléctrica en el lomo sin importar el nivel de estrés previo al sacrificio, pero no pudo compensar los efectos negativos del estrés. Con la excepción en una reflectancia interna aumentada como respuesta a la refrigeración rápida, el color de la carne y otros parámetros asociados a la capacidad de retención de agua no se vieron afectados por el sistema de refrigeración utilizado. Se concluyó que el incremento de la velocidad de refrigeración no es un método adecuado para la resolución de aquellos problemas de calidad de la carne causados por un manejo inadecuado previo al sacrificio.

Se utilizaron los mismos tratamientos de estrés en el estudio referido en el **Capítulo 4**. En dos plantas de sacrificio, que utilizaban, o bien el sistema de aturdido eléctrico o bien el de CO_2, se estudiaron cambios fisiológicos de respuesta al estrés en el período inmediatamente previo al sacrificio y se relacionaron con las características de la calidad de la carne. En ambas plantas, el tratamiento de elevado estrés tuvo como resultado parámetros de calidad de la carne inferiores. El efecto fue más marcado en la capacidad de retención de agua que en el color de la carne, con pérdidas por goteo aumentadas en un 56%. Al contrario que en el estudio referido en el Capítulo 2, la planta de sacrificio con sistema de aturdido mediante CO_2, produjo carne de cerdo con una ligeramente mejor capacidad de retención de agua, en comparación con la planta con el sistema de aturdido eléctrico. De todos los indicadores de estrés medidos en el desangrado, tan sólo el nivel de lactato en sangre estaba fuertemente correlacionado con parámetros de calidad de la carne. Los análisis de regresión revelaron que el nivel de lactato en sangre y el potencial glicolítico explicaban el 52% y el 46% de la variación en las pérdidas de agua por goteo y el valor L* (índice de luminosidad), respectivamente. En combinación con un alto nivel de estrés pre-sacrifico, altos potenciales glicolíticos estaban relacionados con mayores pérdidas de agua. Se concluyó que altos niveles de estrés reducen la calidad de la carne de cerdo, con un efecto agravante de altos niveles de energía en músculo.

En el siguiente experimento (**Capítulo 5**), se investigó si la gran variación en potenciales glicolíticos, encontrada en el estudio anterior, se debía a diferencias en transporte y tiempo de reposo. Además, se estudió si los efectos negativos de altos niveles de estrés, inmediatamente previos al sacrificio, se veían afectados por el manejo

previo en el transporte y el tiempo de reposo. Los cerdos fueron asignados a, bien un transporte corto y suave, o bien un transporte largo y brusco (50 minutos o 3 horas, respectivamente), tiempo de reposo largo o corto (3 horas o <45 minutos, respectivamente) y niveles de estrés mínimo o alto previos al sacrificio. El efecto de los diferentes tipos de tratamiento en el transporte y tiempos de reposo fue mínimo. Se concluyó que, dentro de los rangos utilizados, los tratamientos de transporte y tiempos de reposo, probablemente no eran responsables de la gran variabilidad en potencial glicolítico del músculo. Al respecto de capacidad de retención de agua, por otro lado, la comparación con el nivel de manejo óptimo (transporte corto y suave, largo tiempo de reposo, mínimo estrés) reveló que los efectos negativos de altos niveles de estrés previos al sacrificio, se veían agravados por condiciones de transporte y tiempos de reposos subóptimos. Los niveles de estrés elevados, por si solos, aumentaban la conductividad eléctrica en un 56%, mientras que altos niveles de estrés en combinación con cortos períodos de reposo, llevaban a un aumento del 88%. Sin embargo, el estrés en el período inmediatamente previo al sacrifico, fue el que más contribuyó y el factor más importante en una peor calidad de la carne de cerdo.

En el **Capítulo 6**, se investigó si los efectos de idéntico transporte, tiempo de reposo y tratamientos de estrés dependían del tipo de fibras que componían el músculo. El potencial glicolítico muscular y la calidad de la carne fueron evaluados en el predominantemente glicolítico músculo longissimus dorsi (lomo) así como en el más oxidativo músculo supraspinatus (paleta) o músculo serratus ventralis (culera Boston). Los efectos de condiciones de transporte y tiempo de reposo fueron similares en ambos tipos de músculo. Para el tratamiento de altos niveles de estrés previo al sacrificio, por otro lado, se observaron varias interacciones entre niveles de estrés y tipo de músculo. Los efectos del nivel de estrés en la capacidad de retención de agua eran más marcados en el glicolítico músculo longissimus dorsi, mientras que los efectos sobre el color eran más marcados en el oxidativo músculo serratus ventralis. Se concluyó que la actividad física intensa y/o altos niveles de estrés psicológico previos al sacrifico promueven el desarrollo de la condición PSE (carne pálida, blanda y exudativa) en músculos glicolíticos, mientras que, al mismo tiempo, se incrementa la tendencia hacia DFD (carne oscura, firme y seca) en músculos oxidativos.

En el **Apéndice al Capítulo 6**, los efectos de transporte, niveles de reposo y niveles de estrés se describen exclusivamente al respecto del color en varios músculos de la carne de cerdo (músculos longissimus lumborum, longissimus toracicus y semimembranosus (todos tipos de músculo predominantemente glicolíticos) y el más oxidativo músculo serratus ventralis). El efecto de los diferentes tratamientos de manejo previos al sacrificio difirieron según el músculo estudiado, confirmando los resultados del Capítulo 6. Además existía considerable variación dentro del lomo, con la parte más craneal (longissimus toracicus) más sensible a altos niveles de estrés previos al sacrifico que la parte caudal (longissimus lumborum). Se recomienda evaluar los efectos del manejo previo al sacrificio no en uno sino en varios músculos.

En la **Discusión general**, los resultados de los estudios mencionados se resumen al respecto de la importancia relativa de los factores individuales pre y pos-sacrificio para los atributos de color de la carne y capacidad de retención de agua, así como su efecto en la variación en estos dos atributos. Adicionalmente, se discuten métodos para evaluar el estrés en condiciones prácticas y experimentales así como algunas estrategias para controlar el contenido de glucógeno muscular.

A partir de los resultados presentados en esta tesis se concluye que:

- El estrés en el período inmediatamente previo al sacrificio deteriora el nivel de calidad medio, aumentando asimismo la variabilidad de la calidad de la carne de cerdo. De todos los factores estudiados el nivel de estrés apareció como el más importante.
- El incremento de la velocidad de refrigeración no puede compensar el efecto negativo de altos niveles de estrés.
- Los efectos del método de aturdido utilizado en el color de la carne de cerdo y su capacidad de retención de agua fueron, en comparación con el nivel de estrés, menores y no consistentes en diferentes estudios.
- Las condiciones de transporte y reposo, en los rangos estudiados en esta tesis, tienen poco efecto sobre la calidad de la carne de cerdo. En combinación con altos niveles de estrés previos al sacrifico, sin embargo, condiciones subóptimas de transporte y reposo pueden agravar los efectos de los altos niveles de estrés en el período inmediatamente previo al sacrifico.
- Por si solos, altos niveles de glucógeno muscular en el momento de sacrificio no afectan el color y la capacidad de retención de agua de la carne de cerdo. Sin embargo, en combinación con altos niveles de estrés, altos niveles de glucógeno muscular pueden afectar negativamente a la calidad de la carne de cerdo.

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And who now thinks that these Acknowledgements are too long can maybe imagine what Jaco, Leo, Martin and Coen were faced with when I sent them the first draft manuscripts...

Curriculum vitae

Ellen Hambrecht was born in Freiburg, Germany, on the 6th of October in 1972. After finishing secondary education (Gymnasium) in 1992, she spent one year in a Camphill village in Norway. She worked there with mentally handicapped people mainly on a bio-dynamical farm. Back in Germany, she assisted in an animal hospital in Freiburg for a couple of months before starting her studies of agricultural sciences at the Justus-Liebig-University of Giessen, Germany, in September 1993. From 1995-1996, she worked intermittently 14 months on three different pig and dairy farms. In 1996, she resumed her studies at Hohenheim University, Stuttgart, Germany, where she specialized in animal production. In 1997/1998 she spent 6 months at the Swine Research Centre of Nutreco, Sint Anthonis, the Netherlands. During that period she completed several experiments related to the feeding of weaner pigs. One of these studies formed the basis of her Master thesis titled: 'Effect of non-starch polysaccharides on performance, incidence of diarrhea and gut growth in weaned pigs' which was awarded best agricultural Master thesis of the University of Hohenheim in 1998 and the Schaumann award for animal nutrition studies (1998). She graduated from Hohenheim University in September 1998. In October of the same year, she started her career at the Swine Research Centre of Nutreco in the Netherlands. Also in 1998, pork processor Hendrix Meat Group, a Nutreco company, was formed, and she was assigned pork quality as her research responsibility. This Ph.D. thesis is the result of studies conducted at the Hendrix Meat Group pork processing plants during the years 1999-2003. In 2004, Ellen was transferred to the newly founded Food Research Centre of Nutreco, Boxmeer, the Netherlands, where she will continue to work on pork quality as well as consumer preferences in meat products.

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