ASSESSMENT OF WELFARE OF FINISHING PIGS FROM FARM TO SLAUGHTER

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Preface

This PhD project was part of the Industrial PhD programme, managed by the Innovation Fund Denmark and was conducted in cooperation with Danish Meat Research Institute (DMRI) and Aarhus University (AU). The project involved pigs from commercial private Danish herds who delivered to selected commercial Danish abattoirs. The PhD project was funded by the Danish Agency for Science, Technology and Innovation and the Danish Pig Levy Fund. The PhD project resulted in 3 research papers (PaperI – PaperIII) and 1 review paper (PaperIV). PaperI and PaperIII have been published in Livestock Science and PaperIV in Meat Science. PaperII has been submitted to Animal Welfare.

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Summary
Increasing interest and concerns from the market and the authorities regarding animal welfare have created a need, not only to improve welfare of animals, but also to document the level of animal welfare. The Welfare Quality® Project developed a welfare assessment protocol for pigs on farms and at abattoirs. However, at present no welfare assessment protocol or tools for continuous documentation of animal welfare covering the period from leaving the farm until unloading at the abattoir are available. Hence, the aims of the present thesis were to develop a protocol for welfare assessment of individual finishing pigs from farm to slaughter, to aggregate the welfare measurements into an animal welfare index (AWI), to investigate the relationship between individual behavioural or clinical welfare measurements, the AWIs and post-mortem physiological measurements and to suggest possible candidate measurements for future automation of documentation of welfare based on these relationships.

To fulfil these aims, two studies were conducted under commercial conditions and included 80 and 480 pigs, respectively. A welfare assessment protocol inspired by the Welfare Quality® was developed for the period from farm to slaughter. The welfare measurements were aggregated into an AWI using the weighted linear sum of prevalence on animal level based on expert opinion. In addition to the ante-mortem behavioural and clinical welfare measurements, a blood sample was collected from each pig at exsanguination and analysed for the plasma concentration of glucose, lactate, albumin, total protein as well as creatine kinase activity. In addition, the blood temperature and the post-mortem muscle pH was measured.

The results are presented in three manuscripts, the major results of which were: a) a protocol for welfare assessment of individual finishing pigs from farm to slaughter, b) the aggregation of the welfare measurements included in the protocol into an AWI, in which the selected animal welfare measurements were assigned different weights (PaperII); c) statistically significant, but relatively weak correlations between individual welfare measurements and individual physiological post-mortem measurements (PaperI); and d) between the AWIs and the physiological post-mortem measurements (PaperIII).

In conclusion, the work underlying this thesis has added knowledge to the gap in the assessment of welfare of finishing pigs from farm to slaughter, and led to the establishment of a welfare assessment protocol for finishing pigs in the period from farm to slaughter as well as an aggregated animal welfare index (AWI), which are important tools for future research and welfare assessment. The relatively weak relationships between the AWIs and the post-mortem measurements suggest that exact conclusions cannot be drawn regarding possible candidates for development of a future on-line monitoring of animal welfare on commercial abattoirs. Future research including larger scale studies as well as larger variation in animal welfare is needed.
Sammendrag (Danish summary)


Resultaterne er præsenteret i tre manuskripter. De overordnede resultater var: a) en protokol til velfærds vurdering af individuelle slorgesvin fra besætning til slagtning, b) aggregering af velfærds målene i protokollen til et dyrevelfærdsindeks (AWI), hvor de udvalgte dyrevelfærds mål blev tildelt forskellige vægte (PaperII), c) statistisk signifikante, men relativt svage korrelationer mellem enkelt velfærds mål og individuelle fysiologiske post-mortem mål (PaperI og d) mellem AWI’erne og de fysiologiske post-mortem mål (PaperIII).

Arbejdet bag denne afhandling har tilført viden om velfærds vurdering for slorgesvin i perioden fra besætning til slagtning og har resulteret i etablering af en velfærds vurderingsprotokol for slorgesvin i den pågældende periode, samt et aggregeret dyrevelfærdsindeks (AWI), hvilket er vigtige værktøjer til brug for fremtidig forskning og velfærds vurdering. De relativt svage sammenhænge mellem AWI’erne og post-mortem målene betyder, at der ikke kan drages eksakte konklusioner vedrørende mulige kandidater til udvikling af fremtidig on-line overvågning af dyrevelfærd på kommercielle slagterier. Fremtidig forskning, der involverer et langt større studie med meget større variation i dyrevelfærd, er nødvendigt.
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1. Introduction

Increasing interest and concerns from the market and the authorities regarding animal welfare have created a need, not only to improve welfare of animals, but also to document the level of animal welfare. The Welfare Quality® Project developed a welfare assessment protocol for pigs on farms and at abattoirs. However, at present no welfare assessment protocol covers the period from leaving the farm until unloading at the abattoir. Further, at present, no continuous tool for documentation of welfare during this specific period is available. So far, European legislation prescribes only documentation of stunning effectiveness in the Council Regulation (EC) No. 1099/2009. However, besides stunning, the day of slaughter involves a series of stimuli and events which may negatively affect the welfare of the pigs (Barton, 2004). As examples, previous studies found that repeated regrouping (Coutellier et al., 2007), human handling during moving (Correa et al., 2010; Edwards et al., 2010a), exposure to a novel environment (Lewis et al., 2008), increased stocking density during transport (Warriss et al., 1998) and increased ambient temperature during transport (Sutherland et al., 2009) may pose negative influence on animal welfare (as reviewed in PaperIV). Complete elimination of these events from farm to slaughter is not realistic in a commercial set-up, but their potential negative impact on animal welfare may be limited by appropriate management, and by use of more animal-friendly environments (Barton, 2004).

Under commercial conditions, the typical period from farm to slaughter (the pre-slaughter period) may be split into eight stages each covering one or more of the welfare-relevant events mentioned above: 1) the on-farm pick-up pen; 2) on-farm loading; 3) transport; 4) unloading at the abattoir; 5) lairage; 6) race to the stunning chamber; 7) stunning; and 8) sticking (Figure 1). From an animal welfare perspective, this period terminates at sticking, but welfare relevant measurements may be collected post-mortem on the slaughter line.
Figure 1. The pre-slaughter period can be split into eight stages as illustrated. Irrespective of the time of day, the pre-slaughter period is initiated when pigs are moved from their home pen either directly onto a lorry or to specialised pick-up pens (separate housing units commonly used in Denmark, designed to maximise protection against diseases).

In the future, systematic documentation of welfare from farm to slaughter, as part of daily routines in commercial abattoirs, may be used as a HACCP-like tool to improve the level of welfare by feed-back mechanisms, i.e. if the monitored measurement of welfare suddenly increases above a specified threshold, an alarm could inform the abattoir staff for them to locate and correct the cause for the subsequent batch of pigs. In addition, such documentation can potentially be used for certification, comparison of abattoirs and for marketing (Johnsen et al., 2001).

However, animal welfare is a multi-dimensional concept (Botreau et al., 2007b) as illustrated by the Five Freedoms (Farm Animal Welfare Council, 2009) and the 12 criteria within the large European Welfare Quality Project (Welfare Quality®, 2009). Hence, multiple measurements are needed in order to document animal welfare from farm to slaughter. Systematic documentation of animal welfare requires access to assessment tools and determination of limits for acceptable and unacceptable levels of welfare. However, current knowledge of welfare assessment covering the entire period from farm to slaughter is scarce.
The Welfare Quality Project (WQ®) has developed a comprehensive protocol for an audit based assessment of welfare of pigs (Welfare Quality®, 2009). The WQ® protocols concern either on-farm welfare assessment or welfare assessment at abattoirs, why there is a gap in the knowledge about, and availability of tools for, assessment of welfare of pigs in the interval from leaving the home pen until unloading at the abattoir. The existing WQ® protocol for welfare evaluation in abattoirs has been tested at selected Spanish abattoirs, leading to the conclusion that it is time-consuming (Dalmau et al., 2009) and hence not suitable for systematic documentation purposes under commercial conditions, which need to be relatively quick and cheap. Thus, the development of on-site tools for systematic documentation of welfare covering the entire period from leaving the home pen to sticking, which are applicable at a high commercial slaughter speed, would fulfil the need to cover the entire period from farm to slaughter as well as increase the feasibility of systematic documentation of animal welfare.

In contrast to typical welfare assessment, such as the WQ®, which is based on non-invasive measurements such as behaviour, clinical and resource-based information, other studies have examined potential physiological animal welfare indicators which, if valid, would be more operational for commercial conditions. Examples are findings of relationships between handling prior to slaughter and both exsanguination blood creatine kinase activity (CK) (Correa et al., 2010), whole blood and plasma concentration of lactate (Edwards et al., 2010a; Edwards et al., 2010b; Hambrecht et al., 2004), plasma concentration of lactate and glucose (Becerril-Herrera et al., 2010; Mota-Rojas et al., 2012) and muscle pH 30 minutes after slaughter (Van de Perre et al., 2010). However, knowledge on the relationship between these measurements taken at exsanguination and an aggregated assessment of the entire period from farm to slaughter is scarce. Edwards et al. (2010a) studied the potential relationships between exsanguination blood lactate (LAC) and individual pre-slaughter animal behaviour measurements. They found statistically significant, although modest, correlations between LAC and three animal behaviour measurements (jamming, rearing and backing up), and argued that their findings could potentially be used as a quantitative tool to improve animal handling. If a relationship between an animal welfare index (considered the standard measurement of animal welfare) and one or more physiological post-mortem measurements can be found, the post-mortem measurements may be used as overall welfare approximations. This would be desirable for documentation purposes since selected post-mortem measurements are more likely to become automated than e.g. behavioural or clinical measurements. Moreover, automation of measurements would make systematic
documentation of animal welfare from farm to slaughter possible and have the potential of further achieving the goal of, not only a valid welfare assessment, but also an operational welfare assessment.
2. Aim and hypotheses

The overall aim of this thesis was to add knowledge to the gap in the assessment of welfare of finishing pigs from farm to slaughter under commercial conditions by developing a protocol and an animal welfare index (AWI) for individual pigs. In addition, the aim was to investigate the relationship between individual behavioural or clinical welfare measurements as well as AWIs and physiological post-mortem measurements. The perspectives are to use selected post-mortem measurements as welfare approximations for future automation for documentation of welfare. The overall aim of the thesis was split into two partial aims:

Partial aim I was to develop a welfare assessment protocol for individual finishing pigs from farm to slaughter (PaperI and PaperII) and to aggregate the involved welfare measurements into an AWI for each of 6 stages (from the pick-up pen to the race) and an overall index including all 6 stages by use of expert opinion (PaperII), testing the hypothesis:

- Different animal welfare measurements within stage, as well as the different stages from farm to slaughter, will be assigned different weights in the aggregated welfare assessment.

Partial aim II was to investigate the relationship between physiological post-mortem measurements and individual behavioural or clinical welfare measurements (PaperI) as well as the AWIs, and to suggest possible candidate measurements for future automation of documentation of welfare based on these relationships (PaperIII). This part of the thesis tested the following two hypotheses:

- There is a relationship between selected ante- and post-mortem measurements obtained from pigs from farm to slaughter. E.g. falling in the race will result in increased exsanguination plasma concentration of lactate.

- There is a relationship between an AWI for each of the 6 stages (AWI\text{Stage}) as well as an overall index (AWI\text{Overall}) obtained from pigs in the period from farm to slaughter and selected physiological post-mortem measurements. E.g. the higher the AWI, the higher the plasma concentration of lactate in the exsanguination blood.

To fulfil these aims, two observational studies as well as an online expert opinion survey were conducted. In the present studies, data for the animal welfare assessment were collected under commercial conditions (see details in the material and methods’ section). When conducting observational studies under commercial conditions many factors may interact. Hence, this
study focused at relative, however generalizable conclusions on how the farm to slaughter situation affects the animal welfare of finishing pigs. In Study 1, recordings were performed from stage 4-6 (Figure 1) as well as post-mortem (PaperI). In Study 2, recordings were performed from stage 0 to 6 as well as post-mortem (PaperII and PaperIII). The papers are presented in Chapter 5 of the thesis. The pre-slaughter period (Figure 1) is, in the present thesis, defined as the time span from the pig leaves the home pen until sticking. In Denmark, the pre-slaughter period often begins in the afternoon the day before slaughter, why the pre-slaughter period may range from 2 to 24 hours. In this thesis, the pre-slaughter period is referred to as the period from ‘farm to slaughter’ (in the Papers the period is referred to as ‘the day of slaughter’ independent of the duration).
3. Background

Documentation of welfare of finishing pigs from farm to slaughter requires an assessment of welfare. This chapter introduces the animal welfare definition underlying the animal welfare assessment from farm to slaughter in this thesis, and briefly lists potential measurements of pig welfare from farm to slaughter. Relevant stimuli and events typically present in the period from farm to slaughter, which may function as stressors, and related potential welfare measurements are reviewed in Paper IV. In the review paper we focused on issues relevant for Danish conditions. The last part of this chapter reviews welfare assessment of pigs and methods for aggregation of welfare measurements.

3.1 Definition of animal welfare

Independent of assessment scheme, the conclusion of a welfare assessment or a study involving measurements of animal welfare, depends on the applied definition of animal welfare, which is fundamental for the choice of possible indicators and their interpretation (Sandøe et al., 2012; Veissier et al., 2011). If for example focus is on health as crucial for welfare, a barren environment held as sterile as possible may be preferred, whereas if focus is on the possibility to perform natural behaviour, an enriched environment will receive the better score in terms of animal welfare.

One of the early attempts to define farm animal welfare was the Five Freedoms, introduced in 1979 by the Farm Animal Welfare Council (Farm Animal Welfare Council, 2009). The Five Freedoms are: 1) Freedom from hunger and thirst, 2) Freedom from discomfort, 3) Freedom from pain, disease or injury, 4) Freedom to express normal behaviour, and 5) Freedom from fear and distress. In general, the Five Freedoms provide no information regarding the level of e.g. hunger in order to comply with the definition and no information regarding the assessment of each Freedom relative to the others. As increasing evidence for the sentience of farm animals was documented and incorporated in the European legislation (reviewed by Veissier et al., 2008), there was ongoing discussion within animal welfare science on the importance of the inclusion of different measurements of welfare. Hence, Duncan (1996) argued for an animal welfare definition based on the existence of animal feelings whereas Broom (1996) defined animal welfare “in terms of attempts to cope with the environment”.

One way of distinguishing between different animal welfare views is to classify them as either focusing on function (biological fitness, disease, production etc.), naturalness (the possibility to perform natural behaviours) or emotions (e.g. presence of affective states such as fear, pain or pleasure), however, with a strong opportunity to overlap (Fraser et al., 1997). Fraser et al.
(1997) suggested, that all three views should be considered and within animal science of today, measurements of affective states are acknowledged as important in relation to animal welfare, although the degree of inclusion in different assessment protocols varies, probably because measurements of affective states are difficult to operationalise.

In order to continue the work to develop a general definition of animal welfare, and to operationalise it, the Welfare Quality® Project developed (from 2004 – 2009) an animal welfare matrix consisting of 4 principles ‘good feeding’, ‘good housing’, ‘good health’ and ‘appropriate behaviour’, which were split into 12 criteria: Absence of prolonged hunger, absence of prolonged thirst, comfort around resting, thermal comfort, ease of movement, absence of injuries, absence of disease, absence of pain induced by management procedures, expression of social behaviour, expression of other behaviours, good human-animal relationship and positive emotional state (Welfare Quality®, 2009). Each of these criteria were based on an animal welfare definition focusing on emotions, and was measured by a range of animal welfare measurements.

During the development of the WQ® protocols, a number of different stakeholders were involved such as animal scientists, social scientists, farmers and citizens (Miele et al., 2011). Involvement of stakeholders in the decisions of which measurements to include in a welfare assessment and in the aggregation of these measurements were suggested to be a critical point, since stakeholders may vary considerably in their opinion on which measurements to include. E.g. Sørensen and Fraser (2010) suggested that animal producers and non-producers may have very different opinions about the importance of different factors in relation to animal welfare. For consumers it will typically be important that animals are able to live a natural life (Blokhuis et al., 2003; Lassen et al., 2006), whereas farmers and the food industry may focus more on health (Sørensen and Fraser, 2010).

### 3.2 Welfare measurements from farm to slaughter

As mentioned and illustrated in Figure 1, the period from farm to slaughter contains eight stages in a predetermined order initiated when the pigs leave the home pen. In Denmark, pigs are either loaded directly onto the lorry, or are moved to a pick-up pen before loading. When loaded, the pigs are subject to transport and unloading at the abattoir, where they are kept in lairage until they are moved to stunning and subsequent sticking. Each stage, i.e. pick-up pen, loading, transport, unloading, lairage, race, stunning and sticking, constitutes a novel situation and involves stimuli and events which may act as stressors. These stressors may, individually or in interaction with others, compromise animal welfare. In this thesis, the terms stress and
stressor follow the set of definitions laid out by Sapolsky (2002), emphasising that a stressor is an event, internal or external to the organism, which increases the risk of threatened homeostasis. Stress, then, covers the resultant state of the organism, during which the organism, by use of several types of biological responses, attempts to regain homeostasis. By use of these definitions, stress responses cover physiological responses such as the HPA-axis (Palme et al., 2005), behavioural responses (Jensen and Toates, 1997) and immunological responses (Moberg, 2000), and a state of stress can range from very mild to severe, often with parallel consequences for animal welfare. From farm to slaughter, the potential stressors include among others mixing with unfamiliar conspecifics, aggression, duration of transport and lairage, stocking density and temperature. Details on the potential stressors and related potential welfare measurements are reviewed in PaperIV and summarised in Table 5 of the paper.

As mentioned, animal welfare is a multi-dimensional concept (Botreau et al., 2007b; Botreau et al., 2009) and several aspects related to animal welfare need to be taken into account, as e.g. in the WQ® describing animal welfare including for instance ‘absence of prolonged hunger’, ‘comfort around resting’ and ‘absence of disease’ within a total of 12 dimensions. However, the subjective experiences of animals cannot be measured directly why identification of measurable relevant indicators is necessary (Sandøe and Simonsen, 1992). As an example, recording of wounds and wound size has been suggested as indicators of pain (Sandøe and Simonsen, 1992).

A good indicator of animal welfare constitutes first of all of high validity (relevance for animal welfare) and the selection of indicators for a welfare assessment system is based on weighing validity of measurement candidates against their reliability, operationality as well as feasibility (discussed further in section 6.1.1). Below, behavioural, clinical, physiological and resource-based measurements relevant for welfare in the period from farm to slaughter are listed. Furthermore, the welfare measurements are discussed in PaperIV with regard to relevance and suitability for development of on-site tools for continuous automatic documentation of animal welfare from farm to slaughter. The physiological measurements are only touched upon in this thesis, since their inclusion in the present studies was based on the aim to suggest candidate measurements for future systematic documentation of animal welfare from farm to slaughter, since the post-mortem physiological measurements are considered potentially automatable and hence advantageous as welfare approximations.
**Behavioural measurements**

Behavioural measurements are animal-based, and considered among the most direct welfare measurements, since they include the individual animal, and the underlying motivation is often easier to interpret in terms of welfare, than e.g. physiological measurements.

The period from farm to slaughter is characterised by stages, where pigs are being moved from one point to another (loading, unloading, race to stunning), as well as stages where the pigs are kept in the same environment for a period of time (pick-up pen, during transport and in lairage). Different behavioural measurements of welfare are relevant in the two different conditions, and are treated separately in this section of the thesis. When pigs are being moved, previous studies of animal welfare have included observation of frequencies of slipping, falling, turning back and vocalisations (Van de Perre et al., 2010) as well as reluctance to move forward, attempts to turn, overlapping (Correa et al., 2010), jamming, rearing and backing up (Edwards et al., 2010a) as measurements of welfare. When pigs are kept in the same environment during the stage in question, previous studies have included aggressive behaviour (Barton, 2008; Coutellier et al., 2007; Deen, 2010; Rabaste et al., 2007), mounting behaviour (Thomsen et al., 2012), resting behaviour measured by posture (Rabaste et al., 2007) shivering, panting and huddling (Welfare Quality®, 2009) as measurements of welfare.

In addition to the behaviour of the animals, the behaviour of the handler may provide indirect information of animal welfare such as use of an electric prod (Correa et al., 2010; Grandin, 2012; Hambrecht et al., 2004). Hence, porcine as well as human behavioural measurements are considered relevant for a welfare assessment protocol covering the period from farm to slaughter. The relevance of selected individual behavioural measurements of welfare is reviewed in PaperIV.

**Clinical measurements**

Similar to the behavioural measurements, the clinical measurements are animal-based and refer to health and affective states most often related to injury or pain. Skin damage (Aaslyng et al., 2013; Barton, 2008; Faucitano, 2001; Geverink et al., 1996; Guàrdia et al., 2009) and lameness (Dalmau et al., 2009; Welfare Quality®, 2009) or mortality (Barton et al., 2007; Gosálvez et al., 2006) are well established as clinical measurements of animal welfare. In the WQ® protocol for on-farm assessment of pig welfare, good health is assessed by measurements relevant for the mental state of the animals such as absence of sneezing, coughing, prolapses and infections. In the WQ® protocol for assessment at the abattoir, the criterion 'absence of disease' constitutes of measurements of lameness and dead pigs. In
addition, the WQ® protocol includes measurements obtained at the slaughter line such as pneumonia, but as these measurements reflect the welfare status of the animals on-farm, they are not particularly related to the period from farm to slaughter. Furthermore, in the WQ® protocol for assessment at the abattoir, ‘absence of pain induced by management procedures’ includes stunning effectiveness which can be measured by corneal reflex and nose pricks (Nowak et al., 2007), and as in the WQ® by recording of the corneal reflex, righting reflex, rhythmic breathing and vocalisations (Welfare Quality®, 2009).

Physiological measurements

Two types of stress may occur regarding pre-slaughter handling. Long-term stress occurs as a result of long transport and lairage durations and fast which result in low levels or absence of glycogen in the muscle at the time of slaughter. Short-term stress e.g. caused by use of an electric prod or restraining immediately prior to stunning accelerates the rate of glycolysis (Pearson and Young, 1989). By the time of death glycogen is degraded to glucose and as the oxygen supply is depleted, the glycolysis change to anaerobic glycolysis resulting in lactate (Pearson and Young, 1989). If the pigs are stressed just before stunning, this anaerobic degradation will have started already before slaughter and the lactate concentration in the muscles will be increased (Støier et al., 2001). As long as the blood circulation is intact, the blood will transfer the lactate out of the muscle cells and the concentration in the blood will therefore be increased as well. Furthermore, large variation between individual pigs exists as well as differences in housing, feeding and handling which all may influence the blood levels of glucose and lactate.

In order to use physiological measurements as measurements of reduced animal welfare Jensen and Toates (1997) argued, that interpretation of physiological stress responses needs a motivational context, thereby suggesting that unless documented by behavioural observations or index-scores in the validation phases, physiological measurements are difficult to interpret in terms of welfare. As long as the physiological measurements correlate with animal welfare defined as ‘affective states’, the physiological measurements can, if not alone, then in combination with behavioural and clinical measurements, be relevant to include in a welfare assessment that focus on the mental states of the animals.

One physiological measurement related to the welfare of individual pigs exposed to acute stressors in the period from farm to slaughter is changes in heart rate (Correa et al., 2013; Correa et al., 2010; Lewis et al., 2008). However, heart rate may also increase as a result of
physical activity or arousal why elevated heart rate not unambiguously is negative for welfare and needs consideration when interpreted.

In addition, other physiological measurements have been related to welfare in pigs e.g. the plasma concentration of cortisol (Dokmanovic et al., 2014) and serum concentration of cortisol (Smiecińska et al., 2011; Weeding et al., 1993) as measures of stress, plasma concentration of lactate as a measurement of fatigue (Becerril-Herrera et al., 2010), plasma concentration of glucose (Knowles and Warriss, 2000), total plasma concentration of protein and albumin as measurements of dehydration (Knowles and Warriss, 2000) and plasma creatine kinase activity as a measurement of skin damage (Barton, 2008; Gispert et al., 2000). The relevance of these measurements has been confirmed in previous studies, showing that pre-slaughter handling, resulted in e.g. an increased blood temperature (Hambrecht et al., 2004) plasma concentration of lactate (Correa et al., 2010), whole blood concentration of lactate (Edwards et al., 2010a), serum concentration of glucose (Averos et al., 2007) and serum creatine kinase activity (Mota-Rojas et al., 2009). However, care must be taken when interpreting these values as directly related to animal welfare, as other factors such as hours of last feeding may also influence the metabolism, and hence e.g. the whole blood concentration of glucose which is decreased with increasing hours (Cunningham, 2002). Thus, such factors must be controlled for when possible. Specific relationships are reviewed in detail in Paper IV.

Besides having a negative impact on animal welfare, pre-slaughter handling may also affect meat quality negatively (Gregory, 2008; Støier et al., 2001). Monitoring of animal welfare in the period from farm to slaughter can be used as a tool to optimise welfare, which may contribute to an improved economy due to less off-cutting at the abattoir. Previous studies found that pre-slaughter handling resulted in a decreased pH measured 30-40 minutes after slaughter (Hambrecht et al., 2004; Støier et al., 2001; Van de Perre et al., 2010), which is known to be related to meat quality measured by drip loss (Schäfer et al., 2002).

Resource-based measurements

In addition to the behavioural, clinical and physiological measurements of welfare, resource-based measurements are relevant as indirect indicators of animal welfare. WQ® includes resource-based measurements such as food provision and water supply, flooring and bedding as well as stocking density in the lorry and in lairage (Welfare Quality®, 2009). Other examples of resource-based measurements which may be relevant for the period from farm to slaughter are ventilation/air quality (Sutherland et al., 2009), transport duration (Miranda-de la Lama et al., 2014; Mota-Rojas et al., 2006; Nielsen et al., 2011) lairage duration (Geverink
et al., 1996), ambient temperature (Barton et al., 2007; Schwartzkopf-Genswein et al., 2012; Sutherland et al., 2009), showering (Fox et al., 2014; Nannoni et al., 2013) vibrations of the lorry (Warriss, 1998), manual/automatic handling and group vs. single stunning (Barton, 2004), which may cause a risk with regard to animal welfare.

Compared to the resource-based measurements, the direct animal-based measurements are typically more time-consuming and score low on feasibility, whereas the resource-based measurements can be considered as risk-factors and typically more feasible and reliable (higher inter- and intra-observer reliability), but their validity may be lower (Knierim and Winckler, 2009). The resource-based measurements are useful for identification of risks and causes (Velarde and Dalmau, 2012), for problem solving (Johnsen et al., 2001), and are, thus important to include in a welfare assessment. Often resource-based measurements can provide information needed to solve a problem causing a threat to animal welfare, e.g. if a large percentage of the pigs are falling at unloading, a resource-based measurement can determine whether it is caused e.g. by slippery floors or rough handling.

Previously, welfare assessment schemes included mainly resource-based measurements e.g. TGI35 (Bartussek, 2001) and Freedom Food (Main et al., 2001), since they are more robust and operational compared with direct animal-based measurements. However, animal-based measurements are more direct, since they reflect the state of the animal (Johnsen et al., 2001), and thus are advantageous in terms of validity (Knierim and Winckler, 2009; Webster et al., 2004).

As an example of the potentials and characteristics of the different types of welfare measurements, comparison of welfare assessment using TGI200 (a modified version of the TGI35 (Bartussek, 2001)) and animal-based measurements in 10 dairy herds resulted in two very different results (and low insignificant correlations between the two methods of assessment) (Alban et al., 2001), emphasising that the result of a welfare assessment depends on the chosen measurements. If the assessment aims for high validity as well as high feasibility, it is generally recommended to include animal- as well as resource-based measurements in an overall assessment of animal welfare (Rousing et al., 2001).

### 3.3 Multi-dimensional welfare assessment of pigs

The recent European Welfare Quality Project (Welfare Quality®, 2009) is the most comprehensive initiative towards animal welfare assessment to date. However, the WQ® protocol has not found wide application other than for research purposes. Recently, a reduced version of the WQ® protocol has been evaluated to improve the feasibility, and this version,
which still includes mainly animal-based measurements, has been suggested to provide a satisfactory balance between feasibility and validity (Andreasen et al., 2014). Similarly, experience from the Danish cattle industry, where the Danish Cattle Federation developed a welfare assessment scheme, show that, when the implementation is voluntary and costly, no assessments are carried out, and hence, the scheme does not improve animal welfare (Ingemann et al., 2009).

Common to the mentioned assessment schemes are that they are audit based. Aiming for systematic monitoring of welfare of finishing pigs from farm to slaughter as in the present thesis, feasibility is an important factor, which potentially can be obtained by development of automatic recordings. Physiological post-mortem measurements may be relevant candidates, since, as emphasised by Rushen (2003), they can be used as indicators of welfare when the relationship between a physiological measurement and the underlying motivation is adequately validated.

3.4 Aggregation of welfare measurements
One way to compile the outcome of a welfare assessment into more simple scores are by aggregation of welfare measurements into an index, which allows one score to express the level of welfare, and hence allows comparison of the level of welfare between individual animals and groups of animals based e.g. on simple summations. Methods for aggregation of welfare measurements have been reviewed by Botreau et al. (2007a). One approach is the ‘check-list’ including minimal requirements, where a threshold is set for all measurements included (as in the Freedom Food Scheme). Following this approach means that a farm that fails in one aspect is regarded no different than another farm failing in more aspects. An advantage, however, is that compensation is not possible.

Another method uses the sum of ranks, where a farm score depends on benchmarking to the performance of other farms (Botreau et al., 2007a). Then, the sum of scores results in absolute values (Bracke et al., 2002; Scott et al., 2001), and as such the method allows full compensation (Spoolder et al., 2003), meaning that if a pig receives a low score on e.g. lameness, it can be compensated for in the overall assessment, by a high score on for example disease. To limit compensation, minimum thresholds may be defined (Botreau et al., 2007b; Spoolder et al., 2003).

The Welfare Quality® has suggested a hierarchical aggregation model for animal welfare (Botreau et al., 2007b; Veissier et al., 2011), where the first aggregation step was from measurements to the welfare criteria. Aggregation at this level was based on approximately 5
animal scientists involved in the WQ® project (Botreau et al., 2008). In the second step, the criteria were aggregated to principle scores based on an expert panel including both animal and social scientist opinions, from scientists who were all participating in the project (Veissier et al., 2011). The third step of aggregation into the overall welfare assessment score was based on involvement of other stakeholder groups (Botreau et al., 2008).

The condition of animals can be measured and described, whereas assessment of welfare requires opinions in order to be able to perform an aggregated assessment of animal welfare including decisions on thresholds of acceptable and unacceptable levels of welfare, which is not a part of science as such (Sandøe et al., 2012). Hence, expert opinion is a commonly used method to provide weights for individual measurements when performing overall assessments of animal welfare (Burow et al., 2013; Jensen et al., 2012; Rousing et al., 2007; Welfare Quality®, 2009). Expert opinion can be used at several levels in the construction of an overall animal welfare assessment, e.g. to determine weights for different welfare measurements, to determine limits when categorising measurements, to determine weights for different grades of individual measurements or to decide on acceptable and unacceptable levels of welfare.

Different methods of asking experts to assign weights to selected welfare measurements exist, e.g. ranking of measurements (Lievaart and Noordhuizen, 2011; Whay et al., 2003) and scoring each measurement on a defined scale (Burow et al., 2013; Jensen et al., 2012; Rodenburg et al., 2008; Rousing et al., 2007). Another approach is the Delphi technique, where a panel of participants is initially asked to identify welfare issues as well as animal-based measurements for each issue listed (Whay et al., 2003). Then, the results are used to create a second questionnaire, which is sent to the panellists aiming to obtain consensus (Whay et al., 2003).

As an example, from the WQ®, five experts were asked to rank and score 11 reference farms (theoretical cases) with varying prevalences of lameness from worst to best (Botreau et al., 2008). When selecting this approach, the on-farm prevalence did not linearly reflect the welfare outcome with a slope equal to 1 (i.e. 20% lameness was not scored double as severe as 10% lameness) (Veissier et al., 2011). In contrast to the non-linear reasoning used in the WQ®, linear aggregation models have been proposed by the TGI35 (Johnsen et al., 2001) and by Burow et al. (2013) for transparency reasons. Thus, the results of the use of expert opinion will depend on the questioning method. The use of expert opinion in the present study is discussed in section 6.1.2.
4. Materials and methods

This chapter provides an overview of procedures and methods used in the two studies, Study 1 (PaperI) and Study 2 (PaperII and PaperIII), underlying this thesis. The development of the present welfare assessment protocol was inspired by the WQ® protocol and was initiated in Study 1 (PaperI). Based on these results, the protocol was further developed in Study 2 (PaperII and PaperIII). In addition, this chapter contains elaborated methodological considerations including the process underlying the inclusion and exclusion of measurements in the welfare assessment protocol (the full protocol is available in Appendix section 10.1). Further details regarding materials and methods are presented in the papers (PaperI, PaperII and PaperIII).

4.1 Procedures from farm to slaughter – common for Study 1 and 2

Both studies were designed as observational studies and conducted under commercial conditions with similar procedures from farm to slaughter. Pigs from different herds were included in order to create variation in housing, transport duration, handling etc. and not to test for effect of different housing conditions.

In the present studies, prior to loading, the pigs had been housed in conventional on-farm pick-up pens. Loading and transport were carried out by farm personnel and the commercial hauliers. Upon arrival at the abattoir, all pigs from one lorry were unloaded as a group and moved by the abattoir staff to lairage pens in groups of 15 pigs per pen. After lairage for up to two hours, the pigs were moved by the abattoir staff into a race with automatic push-hoist gates and further into the stunning chamber. The pigs were stunned in groups of 5-8 by use of 90 % CO₂ for three minutes, shackled and stuck according to Danish legislation (Council Regulation (EC) No. 1099/2009). During the two studies, the duration of each of the stages from farm to slaughter was recorded. Data collection was intentionally performed under commercial conditions, and no instructions regarding handling of the pigs were given to farmers or staff.

4.2 Study 1 (PaperI)

The aim of Study 1 was to develop a welfare assessment protocol and to investigate the relationship between individual behavioural and clinical welfare measurements and selected physiological post-mortem measurements.
Animals and herds
A total of 80 pigs from 4 commercial herds were transported by two lorries (pigs from two herds on each lorry) to a commercial Danish abattoir. Further details are described in PaperI.

Data recordings and analysis
Data recordings were carried out at three stages: at unloading, during lairage and in the race to stunning. Recordings of pig behaviour and handling during unloading and in the race were performed using video surveillance. Behavioural recordings at unloading included one-zero sampling of slipping, falling, reluctance to move and turning back. Handling was recorded on a four-point scale, from ‘pig moved voluntarily’ to ‘repetitive strokes with rattle stick, board or hand’. In the race, the recordings included one-zero sampling of slipping, falling, being moved by the automatic gates and lifted by other pigs (overlapping). During lairage, behavioural recordings were carried out by instantaneous sampling and direct observation and included posture and aggression.

The clinical recordings included assessment of skin damage on the shoulder, middle and hindquarters, recorded 45 min after sticking. The physiological post-mortem recordings included blood temperature, plasma concentration of glucose and lactate as well as the plasma creatine kinase activity in the exsanguination blood. After sticking, temperature and pH of m. longissimus dorsi was measured at 45 minutes, and the pH measurements were repeated 22 hours after sticking.

A PCA analysis and a PLSR analysis were used to investigate possible relationships between the ante-mortem measurements of welfare and the physiological post-mortem measurements. The principal component analysis (PCA) can be used as an explorative method to create an overview of large data sets and provides interesting dimensions or directions of variability. The plots can be used for direct interpretation of the results or as a first step before further analyses. The PCA method can handle few missing values, but large number of missing values complicates the interpretation of the results. The method aims to explain as much variance as possible and the interpretation of the results becomes more valid, the higher the explained variance is. The first principal component has the highest possible variance and the subsequent components have the highest possible variance, under the restriction that the component is orthogonal to the preceding component. Variables that are close to each other in the PCA plot have similar properties and variables that are far apart are different from each other (Næs et al., 2010b). The analyses are discussed in section 6.3 in relation to the results of
the studies underlying the present thesis. The partial least square regression (PLSR) maximizes the covariance between linear functions (Næs et al., 2010a).

In addition, pairwise comparisons for specific categorical explanatory variables were performed based on the PCA- and PLSR-analyses. Simple correlations provide knowledge of the covariation between two variables without taking other factors into account. Two common types of correlation coefficients are the Spearman and the Pearson Product-moment correlations. Calculation of Spearman correlation coefficients is used to evaluate relationships including ordinal variables. Pearson Product-moment correlation coefficients are used to evaluate relationships between two continuous variables.

4.3 Study 2 (PaperII and PaperIII)
The aim of Study 2 was to further develop the welfare assessment protocol for individual finishing pigs from farm to slaughter and to aggregate the involved welfare measurements into an AWI for each of 6 stages (from the pick-up pen to the race) and an overall index including all 6 stages by use of expert opinion (PaperII). Furthermore, the aim was to investigate the relationship between physiological post-mortem measurements and the AWIs and based on these relationships to suggest possible candidate measurements for future automation of documentation of welfare (PaperIII).

Animals and herds
The animals of Study 2, underlying PaperII and III, were 480 finishing pigs from 12 different commercial Danish herds (40 pigs per herd). From a subsample of 45 pigs (from five of the herds), heart rate data was recorded and the 45 pigs were included in PaperII aiming to develop the animal welfare index (AWI). In PaperIII, the 480 pigs were included in order to investigate possible relationships between the AWIs and post-mortem measurements. Study 2 was performed at two standard Danish commercial abattoirs. Further details are described in PaperII and PaperIII.

Welfare assessment protocol
The welfare assessment protocol used in PaperII and III was based on the results from Study 1 (PaperI), and consisted of a modified version of the WQ® Protocol. Table 1 shows the WQ® protocol for finishing pigs at an abattoir including the WQ® measurements and a column with the extra measurements included in the present study (referred to as farm to slaughter protocol.
(FTS protocol)) (PaperII and PaperIII). Hence, the FTS protocol included animal-based (behavioural and clinical) as well as resource-based measurements. In addition, physiological measurements were included. The inclusion/exclusion of measurements is elaborated in the following. The overall principles of the construction of the protocol are discussed in section 6.1.

Table 1. The principles, criteria and measurements from the WQ® assessment protocol for pigs (12 criteria) (Welfare Quality®, 2009) and the extra measurements included in the present protocol for assessment of finishing pig welfare, the farm to slaughter (FTS) measurements. A 13th criteria ‘absence of fatigue’ was added to the FTS protocol.

<table>
<thead>
<tr>
<th>Welfare principles, WQ®</th>
<th>Welfare criteria, WQ®</th>
<th>WQ® measurements</th>
<th>FTS measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good feeding</td>
<td>Abundance of prolonged hunger</td>
<td>Food provision</td>
<td>- Albumin, total protein</td>
</tr>
<tr>
<td></td>
<td>Absence of prolonged thirst</td>
<td>Water supply</td>
<td></td>
</tr>
<tr>
<td>Good housing</td>
<td>Comfort around resting</td>
<td>Floor, bedding</td>
<td>Posture, mixing, heat rate, durations</td>
</tr>
<tr>
<td></td>
<td>Thermal comfort</td>
<td>Shivering, panting, huddling</td>
<td>Outdoor temperature</td>
</tr>
<tr>
<td></td>
<td>Ease of movement</td>
<td>Slipping, falling, stocking density</td>
<td>Slipping, falling, Stocking density</td>
</tr>
<tr>
<td>Good health</td>
<td>Absence of injuries</td>
<td>Lameness, wounds on body, Sick animals, dead animals</td>
<td>Lameness, Skin damage, CK, Vet inspection of sick and dead pigs</td>
</tr>
<tr>
<td></td>
<td>Absence of disease</td>
<td>Stunting effectiveness</td>
<td>Overlapping Moved by gate</td>
</tr>
<tr>
<td></td>
<td>Absence of pain induced by management procedures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Appropriate behaviour</td>
<td>Expression of social behaviours</td>
<td>Not applied</td>
<td>Aggression</td>
</tr>
<tr>
<td></td>
<td>Expression of other behaviours</td>
<td>Not applied</td>
<td>- Handling</td>
</tr>
<tr>
<td></td>
<td>Good human-animal relationship</td>
<td>High pitched vocalisations</td>
<td>Reluctance to move Turning back</td>
</tr>
<tr>
<td></td>
<td>Positive emotional state</td>
<td>Reluctance to move, turning back</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Absence of fatigue</td>
<td>-</td>
<td>Glucose, lactate, blood temperature pH</td>
</tr>
</tbody>
</table>

The FTS protocol addressed all four principles of the WQ®, although with some adjustments as described below. The first WQ® criterion ‘absence of prolonged hunger’ was excluded from the FTS protocol. According to legislation, only pigs staying at an abattoir for more than 12 hours are required to be fed (Council Regulation (EC) No. 1099/2009). Since none of the pigs in the present experiments were kept in lairage for more than two hours, none of the pigs were classified as fasting during the study, why prolonged hunger per definition was not relevant. ‘Absence of prolonged thirst’ was measured by albumin and total protein in the
exsanguination blood, as recording of water supply (suggested by WQ®) was not feasible under the given study conditions.

The criterion ‘comfort around resting’ is handled by the WQ® protocol by measurements related to flooring and bedding. The present study was carried out at two Danish commercial abattoirs with similar layouts regarding flooring and use of bedding. Common to both abattoirs, bedding was provided on all lorries, but as all pigs in the study spent less than 12 hours in lairage, no bedding was provided during this period (Council Regulation (EC) No. 1099/2009). Hence, flooring or bedding was not included in the FTS welfare assessment. As an alternative measurement of ‘comfort around resting’, posture during lairage was recorded. Mixing may affect pig welfare negatively (Coutellier et al., 2007), why mixing was initially included in the FTS protocol. However, due to lack of differences in mixing procedures between the 12 herds included in Study 2 (PaperII and PaperIII), mixing did not affect the final index.

Duration is an important characteristic of the stimuli and events present from farm to slaughter, and often important for the consequences in terms of animal welfare, why the duration of each stage from farm to slaughter (FTS) was included in the index. Heart rate is the only continuous measurement included in the present thesis, and heart rate was measured on selected pigs (results presented in PaperII), from their stay in the pick-up pen through to sticking as a measurement of ‘comfort around resting’ (the measurement is described in section 3.2). Driving style of hauliers was recorded in Study 2 by counting obstacles during transport, as driving style has been shown to be an important factor for welfare during transport (Peeters et al., 2008). However, due to lack of variation, the measurement was later excluded from the index.

The fourth WQ® criterion ‘thermal comfort’ includes shivering, panting and huddling. These measurements were not included in the FTS protocol, as postures during lairage and skin damage assessment was prioritised, because these were expected to have a higher prevalence, and due to time constraints, it was not possible to include all these measurements.

For the criteria ‘ease of movement’ and ‘positive emotional state’ the measurements were similar to the WQ® protocol. Similarly, for ‘absence of injuries’, however, extra skin damage assessments were added in the home pen, in the pick-up pen and after lairage in order to cover the entire period from farm to slaughter. In addition, exsanguination blood creatine kinase activity was added as a measurement of muscular effort and tissue damage. ‘Absence of disease’ was checked at the veterinary inspection, but this criterion was not included in the index, as only 1 of 480 pigs showed obvious signs of disease and was humanely euthanised.
upon arrival. Moreover, to be able to fully include the measurement ‘sick animals’ a thorough clinical examination of the pigs would be preferable. ‘Absence of pain induced by management procedures’ was measured by overlapping and moved by gate. Stunning effectiveness is part of the mandatory own-check of the abattoirs and thus regularly examined, why stunning effectiveness was excluded from the present protocol. Similarly, sticking effectiveness was not included in the FTS protocol but was measured automatically at the abattoirs included in the present study by use of the VisStick® system (Borggaard et al., 2011).

‘Expression of social behaviours’ is not included in the WQ® protocol for pigs at abattoirs, but was measured in the FTS-protocol by aggressive behaviour in the lairage pens (PaperIII). Initially, attempts were made to record vocalisations. However, later the measurement was excluded due to lack of ability to distinguish focal pigs under commercial conditions. Handling was recording as a measurement of ‘good human-animal relationship’.

The criterion ‘absence of fatigue’ was added and measured indirectly by the blood temperature and the exsanguination blood glucose and lactate (the measurements are introduced in section 3.2). Indicators of meat quality such as muscle temperature and pH are known to be susceptible to pre-slaughter handling (Støier et al., 2001), why pH45, pH22 and Temp45 were measured in Study 1 (PaperI). However, based on the weak correlations with the ante-mortem variables found in Study 1, Temp45 was excluded from Study 2. Despite the statistically significant correlation of pH22 and falling, overlapping and moved by gate in the race found in Study 1, pH22 was excluded from Study 2, as neither pH or muscle temperature are likely automatic recordings of the future, due to the risk of damaging the meat during sampling. Nevertheless, pH45 was included in Study 2 (PaperIII) to investigate the relationship between the animal welfare index and an indicator of meat quality.

Data recordings and analyses

Data was recorded at seven stages from farm to slaughter (FTS): in the home pen, the pick-up pen, during loading, transport, unloading, lairage and in the race to the stunning chamber. Since the stages differed in aspects such as duration and the involvement of moving of the pigs (stages 2, 4 and 6) vs. pigs being confined to a specific area (stages 0, 1, 3, 5), different welfare measurements were considered important for the different stages. The FTS measurements are listed in Figure 2 according to stage of recording. Direct observation was used when the pigs were stationary (lairage) and video surveillance, when the pigs were being moved from one point to another (loading, unloading, race).
The behavioural recordings at loading and unloading included the frequency of reluctance to move, turning back, slipping and falling. Handling was recorded on a four-point scale during loading and unloading. In the race, the recordings included slipping, falling, moved by the automatic gates and lifted by other pigs (overlapping), and were recorded continuously by counting the number of events at focal pig level. During lairage, behavioural recordings were carried out by instantaneous sampling and included posture and aggressive behaviour (only PaperIII).

The clinical recordings included assessment of skin damage and lameness. Skin damage was scored in the home pen the day before slaughtering, in the on-farm pick-up pen prior to loading, after 1 hour of lairage and on the slaughter line 45 minutes after sticking. Live skin damage assessment was performed in the pens, and lameness was assessed in the home pen by observation of focal pigs.

The physiological recordings included heart rate (from a subsample of the pigs, the results of which are presented in PaperII), plasma concentration of albumin, total protein, glucose, lactate and creatine kinase activity at exsanguination, the exsanguination blood temperature and pH measured in m. longissimus dorsi 45 minutes after sticking. The blood parameters were analysed in plasma, because plasma is more uniform in matrix than serum (Torben Larsen, Pers. comm.).

Possible relationships between the AWIs and the physiological post-mortem measurements was analysed using a linear model with logAWI_{stage} as the response variable and the post-mortem measurements (plasma concentration of lactate, glucose, logck activity, albumin, total protein) as fixed effects (results are presented in PaperIII). pH and blood temperature was analysed similarly using separate models.
Figure 2. The welfare measurements from the farm to slaughter (FTS) protocol split according to the relevant stages from farm to slaughter. Stages 1-6 were included in the calculation of the AWI except for measurements in parenthesis.

Aggregation of measurements into an animal welfare index using expert opinion

An expert panel was invited to provide weights for selected welfare measurements for an aggregated assessment of welfare of finishing pigs from farm to slaughter (FTS) using an online questionnaire (available in Appendix section 10.2). The aim of this work was to get access to a scientific expert opinion of welfare in order to calculate weights to enter into an index, and not to investigate the different opinions within different groups of stakeholders, why the expert panel was composed of experts all having at least a master degree in animal science.

Thirty-nine Danish experts were invited to participate and to add supplementary candidates of relevance. The questionnaire was completed by 24 of the invited experts and by another 15 supplementary candidates. The respondents were employees from Danish abattoirs, universities, the authorities, production consultants, employees from private research centres
and other. Details on recruitment of experts for the panel and distribution of respondents are described in PaperII.

The experts were invited to assign weights for the individual welfare measurements as well as for the individual stages. In connection with questions about loading, unloading and the race to stunning, short video recordings were shown online in order to establish a common basis for answering.

As part of the development of the index, each measurement was transformed into a score of 0 (mild level), 1 (moderate level) or 2 (severe level) independent of recording method, e.g. some measurements were recorded by counting number of events (e.g. slipping, falling, turning back and reluctance to move), some on defined scales (handling, skin damage) and durations were recorded in minutes. The definitions of the categories (0, 1, 2) were based on the literature when possible, stating at which levels the occurrence of a given incidence start to affect the welfare of the animals. When this was not possible, the categorization was done using an empirical inspection of the data. Further information on the specific categorisation is described in PaperII and PaperIII.

The aggregation of the measurements was done using a weighted linear sum of prevalence on animal level, as described by Burow et al. (2013). Details on the calculations are described in PaperII, page 6. The welfare index was calculated relative to a theoretical maximum value within each stage and corrected for the different number of measurements per stage in order not to give more power to criteria containing a higher number of measurements (Botreau et al., 2007b). The index can take on values from 0-100 and the higher the index, the more severe the welfare consequences. When calculating the index in PaperII, the average of the measurement was replacing missing values for the measurement in question. In PaperIII pigs with missing values were excluded.
5. Papers


5.2 PaperII: Brandt, P., Rousing, T., Herskin M. S., Olsen, E. V., Aaslyng, M. D. Development of an Index Based on Expert Opinion for the Assessment of Welfare of Finishing Pigs from Farm to Slaughter. Submitted to Animal Welfare.


5.1 Paper1: Identification of post-mortem indicators of welfare of finishing pigs on the day of slaughter. Published in Livestock Science 157 (2013) 535-544

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Identification of post-mortem indicators of welfare of finishing pigs on the day of slaughter

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ABSTRACT

Welfare measurements inspired by the Welfare Quality® (2009) Protocol for slaughter pigs at the abattoir were recorded for a total of 80 pigs from four herds in order to investigate the relationship between selected ante-mortem observations and post-mortem measurements with the aim of identifying potential welfare indicators for future documentation of welfare of finishing pigs at commercial abattoirs.

Behavioural and clinical observations were carried out at unloading, in the lairage and in the race to the stunning chamber in the abattoir. During lairage, behavioural recordings were performed by direct observation, whereas at unloading and prior to stunning recordings were performed using video surveillance. Behavioural and handling measurements included: reluctance to move, turning back, slipping, falling and driving of the pigs at unloading, postures during lairage and slipping, falling and lifting by other pigs in the race to the stunning chamber. At sticking, a blood sample was collected for analysis of glucose, lactate and creatine kinase activity (CK), the blood and the m. longissimus dorsi (LD) temperature and the LD pH were measured, and skin damages were recorded.

PCA and PLSR analyses were performed to investigate the correlation structures between the ante- and post-mortem measurements. The PCA plot indicated that the plasma concentration of glucose and lactate was correlated with lifting, falling and handling in the race to the stunning chamber, lactate was correlated with pH22, and CK was correlated with skin damage scores. There was a significant relationship between log (CK) and skin damage score on the shoulder ($P=0.0008$).

In conclusion, the study indicated that the plasma concentration of lactate and glucose was correlated with the behaviour and handling in the race to the stunning chamber and might, together with CK, be relevant indicators of welfare of finishing pigs at the abattoir.

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1. Introduction

The day of slaughter includes a number of novel and potentially stressful experiences for the pigs, and it is well known that pigs can show stress responses during this period (Gregory, 2008) which may adversely affect both the welfare of the animals and the meat quality (Gregory, 2008; Støier et al., 2001). Typically, the day of slaughter consists of a complex of potentially stressful elements, the effects of which could be expressed by several different biological outcome measurements relevant for animal welfare (Barton, 2004).

From 2013 onwards, large abattoirs ( > 1000 animal units/yr) in the EU are required to document animal welfare in accordance with an EU Regulation (1099/2009). Compliance with these requirements warrants the development of on-site tools for continuous monitoring of welfare of finishing pigs on...
the day of slaughter, tools that are applicable even at a high commercial slaughter speed.

Recent comprehensive interdisciplinary research in the EU, The Welfare Quality® based on the Five Freedoms defined four principles of animal welfare (good feeding, good housing, good health and appropriate behaviour), together constituting 12 different criteria (Welfare Quality®, 2009) laid down in the current European Standard for welfare assessment of farmed animals. So far, the Welfare Quality® (2009) protocol has been used to assess animal welfare in pig herds (Temple et al., 2011) and in the period between unloading at the abattoir and sticking (Dalmau et al., 2009). However, at present no protocol is available for the evaluation of pig welfare on the day of slaughter from the farm through to sticking.

In this study, we selected measurements inspired by the Welfare Quality® (2009) approach and added several measurements such as blood values, body temperature and meat quality traits, which are not part of Welfare Quality®, but may be suitable for the continuous monitoring of animal welfare required from abattoirs in the future.

Earlier studies available have focused on examination of the consequences of repeated mixing on behaviour and cortisol in saliva (Coutellier et al., 2007), the effects of handling on behaviour, vocalisation, skin damage, heart rate, lactate and the creatine kinase activity (CK) measured in the exsanguination blood (Correa et al., 2010), the effects of handling on the whole blood concentration of lactate (Edwards et al., 2010a, 2010b) and on the plasma concentration of lactate (Hambrecht et al., 2004). In addition, handling associated with loading and slaughter as well as transport of pigs leads to increased levels of lactate, glucose and ear temperature (Becerril-Herrera et al., 2010; Mota-Rojas et al., 2012), to increased creatine phosphokinase (CPK) and lactate (Warris et al., 1994), increased heart rate (Correa et al., 2013, 2010) and increased incidence of skin damage (Mota-Rojas et al., 2006) as well as lower pH 30 min after slaughter (Van de Perre et al., 2010).

Thus, based on these results we selected the level of plasma glucose, lactate and CK in the exsanguination blood as well as skin damage assessment and pH after slaughter as possible physiometabolic and clinical indicators of animal welfare. In addition, we gathered information about inter-herd variation in the selected post-mortem measurements.

The objective of this pilot study was to investigate the relationship between selected ante-mortem observations and post-mortem measurements with the aim of identifying potential welfare indicators for future documentation of welfare of finishing pigs at commercial abattoirs.

2. Materials and methods

2.1. Animals

A total of 80 Danish DLY (Duroc × (Landrace × Yorkshire)) slaughter pigs from four different commercial herds were included in this observational study. The pigs were slaughtered at a live weight of 100–110 kg at approximately 6 months of age. The carcass weight was on average 85.1 kg (± 4.3), 84.1 kg (± 5.6), 75.8 kg (± 2.8) and 85.7 (± 3.6) for herds 1–4, respectively. The pigs were transported in two different commercial three-deck lorries (Finkl, Bissingen, Germany), each of which picked up pigs from at least two herds each day. Prior to loading, the pigs had been housed in conventional delivery facilities (as part of typical Danish practice, the stockman picks pigs from the home pens and house them in delivery pens, which is easily accessible for the lorry driver, for a period before initiation of transport) and 20 pigs from each herd were chosen as focal pigs and numbered individually on the back using a spray marker (either before or after loading, Table 1).

2.2. Study outline and design

This pilot study was designed as an observational study (Kjaer Ersboll et al., 2004) conducted at a standard Danish commercial abattoir (slaughter rate: 820 pigs/h using CO2 group stunning) selected for reasons of convenience. The experiment was conducted in November and December 2011. Since the data collection was based on commercial transports, already scheduled to pick up pigs at several sites each day, the duration of transport differed between herds (Table 1).

All pigs slaughtered on the same day were transported simultaneously on the same lorry and were handled by the same staff at the abattoir. Data recordings were carried out at three checkpoints on the day of slaughter: at unloading at the abattoir, during lairage and in the race to the stunning chamber.

2.3. Procedures on the day of slaughter

The pigs were moved by the stockman from their home pen to conventional delivery facilities (two herds less than 2 h and two herds overnight) according to the normal practice on the farm. The delivery facilities varied from one large pen to smaller pens, each holding 10–13 pigs, however, common to the four herds the pigs were moved without regard to previous pen mates. The pigs were loaded onto a commercial lorry by the lorry driver following normal Danish procedures without regard to previous pen mates, into pens holding between 15 and 23 pigs on the lorry. On arrival at the abattoir, all the pigs from a lorry were unloaded in one large group according to the procedures of the abattoir and were moved by the abattoir staff to the lairage pens in groups of 15 pigs. The focal pigs

<p>| Table 1 |
|---------------------------------|--------|--------|--------|--------|</p>
<table>
<thead>
<tr>
<th><strong>Duration (min)</strong></th>
<th>Herd 1</th>
<th>Herd 2</th>
<th>Herd 3</th>
<th>Herd 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of pigs</td>
<td>110</td>
<td>30</td>
<td>91</td>
<td>55</td>
</tr>
<tr>
<td>No. of focal pigs</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Loading</td>
<td>30</td>
<td>–</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>Transport, total</td>
<td>175</td>
<td>115</td>
<td>120</td>
<td>85</td>
</tr>
<tr>
<td>Break</td>
<td>30</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Unloading</td>
<td>23</td>
<td>23</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Lairage</td>
<td>24</td>
<td>24</td>
<td>53</td>
<td>53</td>
</tr>
</tbody>
</table>

Herd-specific information about the temporal details of the day of slaughter for the 80 pigs involved in the study: duration of loading, transport, breaks, unloading and holding time in lairage.
were distributed among 4–6 pens and pigs from both farms were mixed in the lairage pens. Data collection was intentionally performed under commercial conditions, and therefore no instructions regarding handling of the pigs were provided to the staff. On day 1, the 40 focal pigs were distributed among four adjoining pens, whereas on day 2 the pigs were allocated to six adjoining pens (pen area: 8.6 m², height: 1.06 m solid walls, pigs in adjoining pens).

After lairage, the pigs were driven in groups of 15 out of the lairage pens by the abattoir staff using commercial rattle sticks and into a race with automatic push-hoist gates (Automatic Driveway Systems, Butina A/S, Holbaek, Denmark). The gates moved the pigs towards and into the stunning chamber. The pigs were stunned in groups of 5–6 pigs in 90% CO₂ for 3 min (Backloader, Butina A/S, Holbaek, Denmark), shackled and stuck according to Danish legislation.

2.4. Data recordings

2.4.1. Temperature

During transport (day 1) and lairage (days 1 and 2), the ambient temperature was logged every 5 min at the deck partition in the lorry and at the pen partition in the lairage, respectively (Tinytag Ultra 2, Gemini Data Loggers (UK) Ltd., Chichester, West Sussex, United Kingdom). The average temperature was 14.8 ± 1.8 °C in the lorry on day 1, and 13.5 ± 0.3 °C and 14.9 ± 0.7 °C in lairage on days 1 and 2, respectively.

2.4.2. Behavioural recordings

2.4.2.1. Video surveillance. The behaviour of the pigs during unloading and in the race leading to the stunning chamber was recorded using video surveillance (MONACOR, TVCCD-1 40IR, Bremen, Germany). Some video sequences were observed once in order to define the ethogram (Table 2). When all the video sequences had been analysed for pig behaviours and handling, the observations and scores were verified by a second observation. One trained observer performed all recordings from the video surveillance.

2.4.2.2. Handling during unloading from the lorry. Scoring of the behaviour of the staff and pigs was initiated at the ramp of the lorry and ended when the pigs had passed the visual veterinary inspection just before entering the lairage area. The handling of the animals during unloading was recorded continuously by focal animal one-zero sampling (Martin and Bateson, 2007) using video surveillance (MONACOR, TVCCD-1 40IR, Bremen, Germany) and a scale ranging from 0 to 3, in which 0 = the pig moves voluntarily or is driven along by the herd; 1 = the staff used a rattle stick or board (just touching); 2 = the staff used a rattle stick, board or hand (single stroke); or 3 = the staff used a rattle stick, board or hand (repetitive strokes). For each pig, the highest score (0–3) obtained during the process was used for further analysis.

2.4.2.3. Pig behaviour during unloading from the lorry. On arrival at the abattoir, the behavioural recordings during unloading included one-zero sampling of reluctance to move, turning back, slipping and falling for all focal pigs (Table 2).

2.4.2.4. Handling in the race. In the race to the stunning chamber, it was recorded whether the pigs were physically moved by the automatic gates (the pig was pushed forward by the automatic gate) or whether the pigs were lifted by other pigs due to lack of space (the pig was lifted by another pig, where at least one leg of the pig being lifted was not in contact with the floor in the race). The recordings were performed continuously using video surveillance (MONACOR, TVCCD-1 40IR, Bremen, Germany) and focal animal one-zero sampling (Martin and Bateson, 2007).

2.4.2.5. Pig behaviour during lairage. Behavioural observations during lairage were conducted using instantaneous sampling and direct observation (Martin and Bateson, 2007), and each pen was observed by one trained observer for periods of 2 ¼ min separated by 10–15 min intervals. At the initiation of each observation period, the posture of each focal pig was recorded (standing, sitting or lying), and during the observation period the occurrence of aggression was recorded (Table 2).

<table>
<thead>
<tr>
<th>Checkpoint</th>
<th>Behaviour</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>u, r</td>
<td>Slipping</td>
<td>The pig slips on at least one leg, the body does not come into contact with the floor</td>
</tr>
<tr>
<td>u, r</td>
<td>Falling</td>
<td>The pig loses balance on one or more legs, and falls to its knees, or a part of the body other than legs comes into contact with the floor</td>
</tr>
<tr>
<td>u</td>
<td>Reluctance to move when being driven</td>
<td>The pig stops for &gt; 2 s (Correa et al., 2010) while being driven forward by rattle stick or board</td>
</tr>
<tr>
<td>u</td>
<td>Turning back when being driven</td>
<td>The pig turns more than 90° away from the direction of movement while being driven forward by rattle stick or board</td>
</tr>
<tr>
<td>l</td>
<td>Posture during lairage</td>
<td>Lying: the body of the pig is in contact with the floor, either sternally, laterally or combined Sitting: the pig rests on its thighs and front legs (Tertoux et al., 2009) Standing: the pig is supported on all four legs</td>
</tr>
<tr>
<td>l</td>
<td>Aggression</td>
<td>Two or more pigs interact with agonistic behaviour, including threats made by a single pig and mutual interactions. Both the maker and the recipient of the threat were recorded: head knocks; rapid sideways movements of the head directed at another pig (Barton Gade, 2008) or biting; one pig snaps at or bites another pig (Barton Gade, 2008)</td>
</tr>
</tbody>
</table>
2.4.2.6. Pig behaviour prior to stunning. In the last three compartments prior to the stunning chamber, the behaviour of the pigs was recorded by video surveillance (MONACOR, TVCCD-1 40IR, Bremen, Germany) with the camera positioned next to the race. The occurrence of slipping and falling was recorded (Table 2) by one observer using one-zero sampling (Martin and Bateson, 2007).

2.4.3. Clinical recordings
Skin damage on the shoulder, the middle and the ham was recorded 45 min after stunning, using the anatomical locations and procedures described by Barton Gade et al. (1996). One experienced observer performed all examinations (slaughter line speed: 410 carcasses/h). A four-point scale was used, in which 1 = no skin damage; 2 = slight skin damage; 3 = skin damage affecting quality; and 4 = extreme skin damage with possible rejection of tissue (Barton Gade et al., 1996). The highest scoring body side of each carcass determined the score.

2.4.4. Physiological recordings
At exsanguination, the blood temperature was measured using a portable thermometer (Testo 720, Testo AG, Lenzkirch, Germany) placed in the sticking wound for at least 3 s. Blood samples were collected in heparin-coated tubes (VACUETTE®, 9 ml NH Sodium Heparin 16 × 100, Greiner Bio-One GmbH, Kremsmünster, Austria) and stored on ice until centrifugation (2000 rpm for 10 min) within 4 h of sampling. Plasma was stored at −18 °C until analysis within eight weeks. The plasma samples were analysed for glucose, lactate and CK (automatic biochemical analyser, ADVIA® Chemistry Systems, Siemens, Erlangen, Germany). All intra- and inter-assay precisions were within 4% (CV).

The temperature (Testo 106-T2, Testo AG, Lenzkirch, Germany) and pH (Portamess® 913 (X) pH, Knick, Berlin, Germany) were measured in the m. longissimus dorsi (LD) at the 4–5th lumbar vertebrae 45 min (Temp45 and pH45, respectively) after stunning. The pH measurements were repeated 22 h after stunning (pH22).

2.5. Statistical analysis
Before analysis the CK values were log-transformed to meet requirements for normal distribution. Values below 34 °C for blood temperatures were considered outliers and were removed before analysis (one sample, corresponding to 2%, was excluded). Due to very low frequencies of aggression, the recordings were not included in the data analyses.

Even though this study was not designed to compare individual herds, variation in the response variables, for example between herds, would provide information which can be used to differentiate between levels of welfare. To test for differences between herds, the response variables were analysed using a linear model:

\[ Y_i = \mu + h_i + e_i \]

where \( Y_i \) was glucose, lactate, log(CK), blood temperature, temp45, pH45, pH22 or skin damage score at the shoulder, middle and ham as well as a summarised skin damage score (s-score) \( ((\text{shoulder} + \text{middle} + \text{ham})−3) \), respectively. Herd \( (h) \) \( (i = 1, 2, 3, 4) \) was included as a fixed effect. \( e_i \) was assumed to be independent and normally distributed. Due to a significant difference between herds in a number of the response variables, herd was included as a random effect in the subsequent analyses.

A PCA analysis (Unscrambler®, Camo Software AS, Oslo, Norway, ver. 9.8) was carried out including ante-mortem and post-mortem measurements. In addition, a PLSR analysis (Unscrambler®, Camo Software AS, Oslo, Norway, ver. 9.8) was carried out to create a correlation plot combining the post-mortem and ante-mortem measurements. Pearson’s product–moment correlations were calculated.

Based on the information from the PCA and the PLSR plots, pairwise comparisons for specific categorical explanatory variables were carried out. The relationship between log(CK) and skin damage scores (shoulder, middle and ham) was analysed using a linear mixed model:

\[ Y_{ijkl} = \mu + s_i + m_j + a_k + H_i + e_{ijkl} \]

where \( Y_{ijkl} \) was log(CK), and skin damage score on the shoulder \( (s) \) \( (i = 1, 2, 3, 4) \), middle \( (m) \) \( (j = 1, 2, 3, 4) \) and ham \( (a) \) \( (k = 1, 2, 3, 4) \) were included as fixed effects. Herd \( (H) \) \( (i = 1, 2, 3, 4) \) was included as a random effect Herd\( \sim N(0, \sigma_H^2) \). \( e_{ijkl} \) was assumed to be independent and normally distributed. Non-significant effects were removed by backward elimination, and least squares means were estimated and reported together with the P-values.

Similarly, the relationship between the plasma concentration of lactate and pH22 was analysed using a linear mixed model where herd was included as a random effect. The significance level used was 0.05. The statistical analyses were carried out using R (R Development Core Team, 2011) and, in particular, the extension packages “lme4” and “multcomp”.

3. Results
The results of this study are presented chronologically below. Table 1 summarises the details of the transports from the four involved herds, and the description of the behaviour and handling of the pigs at the three checkpoints is shown in Table 3.

3.1. Unloading
Measurements of driving of finishing pigs at the abattoir were collected at unloading. Most pigs received a driving score of zero at unloading (77%) and only two pigs received score 3 (1%). 8% And 14% received score 1 and 2, respectively. At this checkpoint 6% of the pigs were observed slipping and 4% falling (Table 3).

3.2. Laiage
The time spent in lairage was in accordance with the abattoir routines and differed between the two days (Table 1). During lairage, the postures of the pigs were recorded in 10–15 min intervals. The occurrence of the different postures at the 1–3 observations performed per herd is presented in Table 3. Initially, 50–75% of the pigs
were observed lying down, increasing to 80–100% of the pigs at the next observation, performed 10–15 min later apart from herd 2 where, only 20% of the pigs were lying down at this time point. In total, only few pigs were observed sitting.

3.3. Race

The behaviour of the pigs in the race to the stunning chamber is shown in Table 3. At this checkpoint 3% of the pigs were observed slipping and 11% falling. 56% Of the pigs were moved by the automatic gate.

3.4. Post-mortem measures

Table 4 summarises the post-mortem measurements stratified by herd. The blood temperature (P < 0.0001) and pH (pH45: P = 0.03 and pH22; P = 0.02) varied significantly among herds. Furthermore, the herds differed significantly with regard to the plasma concentration of lactate (P = 0.01) and log(CK) (P < 0.0001), but not the plasma concentration of glucose. Herd 3 in particular had higher blood values compared with the other herds.

Furthermore, Fig. 1 shows a significant herd-dependent difference (P < 0.005) in the prevalence of skin damage assessed on the slaughter line. Herd 3, which had high blood values, also had high skin damage scores. However, the latter was also found for herd 1.

3.5. Correlations between ante- and post-mortem measurements

The PCA plot (Fig. 2) shows that the plasma concentration of glucose and lactate was related with events in the race to the stunning chamber. A simple correlation analysis showed that the plasma concentration of glucose was correlated to Slip_r (r = 0.26, P = 0.02), Gate_r (r = 0.26, P = 0.02), Lift_r (r = 0.09, P = 0.4) and Fall_r (r = 0.19, P = 0.09) and plasma concentration of lactate was correlated to Slip_r (r = 0.15, P = 0.2), Gate_r (r = 0.18, P = 0.1), Lift_r (r = 0.10, P = 0.4) and Fall_r (r = 0.13, P = 0.3). In addition, lactate was negatively correlated with pH45 (r = −0.14, P = 0.2) and pH22 (r = −0.18, P = 0.1). pH22 was positively correlated to Lift_r (r = 0.22, P = 0.05), Gate_r (r = 0.30, P = 0.007) and Fall_r (r = 0.30, P = 0.008) in the race to the stunning chamber. The plasma concentration of log(CK) was significantly correlated with skin damage scores (shoulder: r = 0.47, P < 0.0001, middle: r = 0.26, P = 0.02 and ham: r = 0.26, P = 0.02) and the relationship between log(CK) and skin damage was investigated using a mixed model. A significant positive relationship was found between skin damage score on the shoulder and log(CK) (P = 0.0008) (Fig. 3). No significant relationships between log(CK) and the other skin damage scores were found, even though data indicated the same trend (data not shown).

Based on the results of the PCA analysis, the relationship between the plasma concentration of lactate and pH22 was examined using a mixed model. However, no significant relationship was found (results not shown).

The PLSR plot (Fig. 4) shows a correlation between events in the race and lactate meaning that if the pigs were slipping, falling or moved by the gate, the content of lactate in the sticking blood would be higher. Furthermore, an opposite relationship was seen between the Posture2
Table 4
Post-mortem measurements taken from the pigs at exsanguination and 45 min and 22 h later. Data are stratified by herd and presented as LS means (S.E.).

<table>
<thead>
<tr>
<th>Herd</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood temp (°C) (n=51)</td>
<td>39.4 (0.10)a</td>
<td>39.2 (0.15)a</td>
<td>38.6 (0.10)b</td>
<td>39.1 (0.10)a</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Glucose (mM) (n=80)</td>
<td>15.7 (1.02)</td>
<td>17.2 (1.02)</td>
<td>18.0 (1.02)</td>
<td>16.3 (1.02)</td>
<td>NS</td>
</tr>
<tr>
<td>Lactate (mM) (n=80)</td>
<td>7.2 (0.40)ab</td>
<td>7.3 (0.40)ab</td>
<td>8.2 (0.40)ab</td>
<td>6.3 (0.40)b</td>
<td>0.01</td>
</tr>
<tr>
<td>log(CK) (mM) (n=80)</td>
<td>8.2 (0.18)a</td>
<td>8.1 (0.18)ab</td>
<td>9.5 (0.18)ab</td>
<td>7.7 (0.18)b</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>CK (U/L) (n=80)</td>
<td>4890 (1781)a</td>
<td>4510 (1781)a</td>
<td>18269 (1781)b</td>
<td>3786 (1781)a</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Temp45 (n=78)</td>
<td>39.4 (0.09)</td>
<td>39.5 (0.09)</td>
<td>39.2 (0.09)</td>
<td>39.2 (0.09)</td>
<td>0.07</td>
</tr>
<tr>
<td>pH45 (n=78)</td>
<td>6.8 (0.40)</td>
<td>6.8 (0.40)</td>
<td>6.7 (0.40)</td>
<td>6.8 (0.40)</td>
<td>0.03</td>
</tr>
<tr>
<td>pH122 (n=78)</td>
<td>5.53 (0.02)b</td>
<td>5.47 (0.02)b</td>
<td>5.54 (0.02)bc</td>
<td>5.55 (0.02)bc</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Different letters (a and b) indicate a significant difference at P < 0.05 in each row.

Fig. 1. The relative skin damage score (s-score) collected from the pig carcasses at the slaughter line, summarizing the scores from the shoulder, middle and ham (n=77), varying from 0 to 9. No carcass received a score above 6. Data are stratified by herd and presented as LS means (S.E.), and different letters (a and b) indicate a significant difference at P < 0.005.

Unfortunately, it was not possible to use the current behavioural observations to distinguish between the underlying motivational states of the lying pigs. Heart rate measurements have previously been used to distinguish stress responses in pigs (Correa et al., 2013, 2010), and behavioural observations combined with heart rate measurements could facilitate the interpretation of the motivational state of the pigs during lairage and other phases of the day of slaughter. Therefore, to interpret the animals’ underlying motivational state in future studies, it would be advantageous to include measurements such as heart rate, albeit the potential influence of the equipment on the behaviour of the pigs. However, a low level of activity in the lairage pens independent of the underlying motivation appears to be a welfare advantage, since the risk of the pigs causing damage to each other will be reduced. Only CK of the selected physiological measurements correlated significantly with the occurrence of lying behaviour in the lairage in this study although the correlation was small (r = 0.24), why other measurements probably will be needed in order to document animal welfare at this stage. The occurrence of aggressive behaviour was recorded during lairage, although the occurrence of the behaviour was very low frequent, and therefore not included in the analyses.

4. Discussion

The present study examined relationships between selected potential welfare relevant measurements obtained from live animals and carcasses of finishing pigs at a commercial Danish abattoir. The study showed a significant positive correlation between the creatine kinase activity (CK) in the exsanguination blood plasma and skin damage score on the shoulder examined 45 min after stunning. In addition, the plasma concentrations of lactate and glucose were correlated with behaviour and handling in the race to the stunning chamber. Based on one sample from the exsanguination blood, these post-mortem physiological measurements might be relevant indicators of welfare of finishing pigs on the day of slaughter, and be suitable for future documentation of welfare at abattoirs. Thus, we suggest that they should be potential candidates to focus on in future studies of welfare on the day of slaughter.

During lairage there were no clear correlations between behaviour and the physiological responses obtained post-mortem. The PCA and PLSR analyses indicated negative correlations between time spent in different postures and log(CK) (r = 0.24). However, it is important to mention that the fact that the pigs lied down during lairage may reflect at least two different motivational states of the animals: the pigs may lie down either to relax in comfortable surroundings or due to exhaustion after moving, mixing, transport etc.
Other studies have reported levels of plasma CK around 465 U/L before transport and 5000–6000 in the exsanguination blood (Mota-Rojas et al., 2009) and serum CK of 2337 before loading, 7698 at unloading and 6058 U/L at exsanguination (Averos et al., 2007), compared to plasma CK between 3786 and 18,269 U/L in the exsanguination blood in the present study. Correa et al. (2010) reported CPK values in the exsanguination blood around 11,800 U/L when comparing different moving devices at loading and Warriss et al. (1994) reported values of 965 U/L in low-stress systems and 1436 U/L in high stress systems. There is, thus, a large variation of CK levels between studies. The values found in this study are above previously found baseline levels (3800–4900 U/L), particularly for herd 3 (18,269 U/L). Since the transport duration (up to 175 min) and lairage duration (up to 53 min) was short and the CK rise for 6 h, the CK levels in the exsanguination blood probably reflects handling and skin damage that occurred on farm.

The body temperature of a pig early post-mortem reflects the physiological state of the animal just before stunning (Barton, 2004), since an aroused animal will have an increased metabolic rate and a higher body temperature. A previous study found blood temperatures varying between 35.6 and 42.6 °C and ear temperatures varying between 27.3 and 35.0 °C immediately after sticking and found a positive correlation of 0.71 between the two temperature measurements at group level (Warriss et al., 2006). Furthermore, the study showed a positive correlation between CK and body surface temperature of 0.55 and a positive correlation between CK and blood temperature of 0.38 (Warriss et al., 2006). Due to such positive correlations, one of the measurements may be sufficient for future documentation purposes. In the present study, it was not possible to demonstrate a positive correlation between CK and blood temperature ($r = -0.19$), even though the temperatures were in the same interval as reported by Warriss et al. (2006). This lack of correlation could be due to too few blood temperature measurements in the present study.

A previous study found a positive correlation between exposure to an acute stressor (an electric prod) and blood temperature in pigs in an abattoir where electrical stunning was used, but did not find this relation in an abattoir using CO$_2$ stunning, however, without discussing this difference (Hambrecht et al., 2004). The authors reported that 30 min after slaughter the loin temperatures were significantly higher in the stressed pigs at both abattoirs, but that this difference was larger in the abattoir using electrical stunning (Hambrecht et al., 2004). In the present study, it was not possible to demonstrate a relationship.
between behavioural consequences of handling and blood or loin temperatures, which might be due to a difference in methodology in the temperature measurements or that the handling has not been sufficiently rough to cause a state of stress and stress-induced hyperthermia. Thus, blood temperature has previously been used as an indicator of pre-slaughter stress when using electrical stunning. The blood temperature may be related to an overall score of pre-slaughter welfare, since an overall score may cause larger variation in the pre-slaughter measurements compared to testing for correlations between single measurements as in the present study.

Our study showed a positive correlation between handling in the race (slipping, falling, lifting and moved by gate) on the plasma concentration of glucose and lactate, where the exposure to the recorded events in the race led to increased concentration of glucose and lactate in the exsanguination blood, although the correlations for lactate was insignificant. Similarly, previous studies found that the exsanguination blood concentration of lactate was related to handling prior to slaughter (Edwards et al., 2010a; Hambrecht et al., 2004; Warris et al., 1994). In addition, transport durations of 8 and 16 h resulted in significantly increased blood glucose, lactate and haematocrit levels compared to baseline measures in Pietrain LY crosses (Becerril-Herrera et al., 2010). Plasma lactate has been shown to increase to its maximum within 4 min after physical exercise and return to basal level in 2 h (Anderson, 2010). Mota-Rojas et al. (2012) reported a baseline measurement of lactate of 32–34 mg/dL (corresponding to 3.6–3.9 mmol/L) and between 75–100 mg/dL (corresponding to 8.6–11.2 mmol/L) in exsanguination blood and Mota-Rojas et al. (2009) reported a plasma lactate concentration of 5.1 mmol/L before transport and 24.67 mmol/L after 118 km of transportation. In the present study the exsanguination plasma lactate concentrations was between an average of 6.3 and 8.2 mmol/L and there was positive correlations between plasma lactate in exsanguination blood and handling in race. These results suggest that the plasma lactate was increased above baseline levels and combined with a rapid increase in lactate concentration, the increased levels of lactate is most likely due to the handling and behaviour in the race to the stunning chamber.

In addition to the blood measurements, pH22 was positively correlated with events in the race (Lift_r: r=0.22, Gate_r: r=0.30 and Fall_r: r=0.30). A previous study showed a significant decrease in muscle pH 30 min post-mortem and a significant increase in ultimate muscle pH for pigs subjected to high pre-slaughter stress (Hambrecht et al., 2004). A recent study found no relationship between handling prior to slaughter and pH one hour post-mortem in the LD or in the m. semimembranosus and no significant relationship between handling and ultimate pH in the LD (Correa et al., 2010). However, the authors reported a significant increase in ultimate pH in the m. semimembranosus and m. adductor when using an electric prod compared to compressed air prod or paddle (Correa et al., 2010). Thus, pre-slaughter stress may affect the ultimate muscle pH, whereas results obtained 30 min to one hour post-mortem show less clear differences in pH, which is similar to the present study. Ultimate pH may thus constitute an indicator of welfare, although the ultimate pH is unlikely to be feasible as an online measurement for documentation of welfare.

The duration of transport have been shown to affect welfare of pigs and meat quality traits where transport duration of 8 h resulted in increased number of pigs with pH 45 min after slaughter below 5.7 compared to transports of 16 and 24 h and an increased number of pigs with pH above 6.3 when transported for 24 h compared to an
8 h transport duration in Pietrain LY crosses (Mota-Rojas et al., 2006). In this study pH45 was not correlated to the handling and behaviour at the slaughterhouse. There was a small, but significant, difference between herds in pH45 (average 6.7–6.8), but the herd with the lowest average pH was a herd with a medium transport time. The longest transport duration was, however, only 175 min in this study (Table 1) and this might not be enough to induce the effects in pH described in the other studies using transport durations above 8 h.

A previous study focusing on stress measurements from blood serum of finishing pigs showed increased concentrations of CPK, lactate dehydrogenase (LDH), total protein, albumin and cortisol from loading to unloading at the abattoir, and decreased values were found between unloading and exsanguination after six hours of lairage (Averos et al., 2007). The authors suggested that lairage allowed the physiological measurements to decrease (Averos et al., 2007). Furthermore, Mota-Rojas et al. (2009) found blood CK activity, glucose, lactate and albumin values increased significantly between loading and unloading and that glucose, lactate and albumin concentrations were significantly higher in pigs subjected to one hour of lairage, whereas CK was higher in pigs subjected to five hours of lairage. At exsanguination, glucose, lactate, albumin and CK were all higher after only one hour of lairage (Mota-Rojas et al., 2009). Thus, a limited period of lairage, as in the present study where the pigs spent 24–53 min in lairage, probably does not allow for the blood values to decrease, supporting their relevance as indicators of pre-slaughter stress. However, in the present study we did not aim to separate effects of pre-slaughter handling at unloading and lairage, and thus only exsanguination blood was sampled to identify potential post-mortem indicators of pre-slaughter welfare.

Besides being exposed to pre-slaughter handling procedures, pigs are typically physically active and often fasted on the day of slaughter (Barton, 2004). The level of physical activity and fasting can affect the blood measurements, and thus influence the interpretation – for example walking a longer distance may result in lower blood glucose, which is not necessarily a threat to welfare compared with exhaustion caused by mixing or rough handling. However, when identifying post-mortem measurements for future automatic recordings, this information will not be available and the post-mortem measurements will only identify changes in the level and not the cause. If the aim is to identify the underlying cause of an increased or decreased level of a certain blood measurement, alternative experimental designs must be used, e.g. involving baseline measurements and interventions.

To be able to assess the relationship between the ante-mortem behaviour and handling measurements with post-mortem measurements, an aggregation of the measurements is desirable, since each of the observed behaviours alone might not introduce a variation, whereas together they might influence, for example, the blood lactate concentration. Aggregation of multiple welfare-related measurements is not necessarily explicit, and the relative contribution of the different measurements should be considered when evaluating the overall welfare (Botreau et al., 2007). However, no model for the aggregation of measurements is currently available, and therefore the analyses in this study are based on single measurements only.

5. Conclusion

This pilot study indicated that the occurrence of certain types of behaviour (slipping and falling) and handling (pig moved by gate or lifted by other pigs) in the race to the stunning chamber prior to slaughter at a commercial abattoir correlated positively with the plasma concentration of glucose and lactate, while the creatine kinase activity correlated positively with skin damage on the shoulder assessed on the slaughter line. Thus, these post-mortem measurements might be potential indicators of welfare, to be used for documentation of animal welfare of finishing pigs on the day of slaughter. Future studies are needed to reveal whether the post-mortem measurements can be used to reflect the overall welfare of the pigs on the day of slaughter and whether these measurements can be automated under commercial conditions.

Conflict of interest statement

None of the authors have any conflict of interest to state.

Acknowledgements

The authors gratefully acknowledge the employees at Danish Crown and Dansk Grisetransport and Maiken Baltzer, Peter Vorup, Lars Ole Blaabjerg and Helle Daugaard Larsen for assisting in conducting the experiment, and the Danish Agency for Science, Technology and Innovation and the Pig Levy Fund for funding the project.

Reference


5.2 PaperII: Development of an Index Based on Expert Opinion for the Assessment of Welfare of Finishing Pigs from Farm to Slaughter. Submitted to Animal Welfare (19th November 2015)

Included in the thesis with permission from UFAW.
Development of an Index Based on Expert Opinion for the Assessment of Welfare of Finishing Pigs from Farm to Slaughter

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Abstract

For pigs, the day of slaughter involves a series of potential stressful elements. Based on the Welfare Quality® protocol for finishing pigs at an abattoir, we developed a welfare assessment protocol as well as an animal welfare index (AWI) for finishing pigs from leaving the farm to slaughter. AWIs were calculated on animal level as the weighted sums of prevalence of a total of 25 measurements. AWIs were calculated for each of the six stages, AWI\textsubscript{Stage} (stages: pick-up pen, loading, transport, unloading, lairage and race) and across the stages (AWI\textsubscript{Overall}). Expert opinion (38 experts from six affiliations) was used for providing weights for the aggregation of measurements. No significant effects of expert affiliation on weighting of the scores were found. There were, however, significant differences between the mean expert scores in all stages for the individual welfare measurement within stage, but no difference between stage weights.

To evaluate the developed AWI, a welfare assessment was carried out including a total of 45 pigs from five commercial herds and involved behavioural (e.g. turning back and reluctance to move) and clinical health measurements (skin damage and lameness). The average heart rates within each stage were 123\textsuperscript{a}(pick up pen), 139\textsuperscript{b}(loading), 120\textsuperscript{a}(transport), 132\textsuperscript{d}(unloading), 114\textsuperscript{c}(lairage) and 134\textsuperscript{bd}(race) bpm, respectively, and significant differences in average heart rates between stages were found (different letters indicate a significant difference at $P<0.05$). There was no relationship between the score per stage assigned by the expert panel and the average heart rate within stage.
(r=0.61, P=0.19) or AWI\textsubscript{Stage} and heart rate (r=0.43, P=0.40). When lairage was removed, the correlations were (r=0.95, P=0.01) and (r=0.84, P=0.08), respectively.

In conclusion, a welfare index within single stages may be useful for intra-stage comparison between days, abattoirs etc., but will be less useful for comparison between stages.

*Keywords*: animal welfare, expert opinion, finishing pig, heart rate, welfare assessment, welfare index
Introduction

Increasing interest and concerns from the market and the authorities regarding animal welfare have created a need, not only to improve the welfare of animals, but also to document the level of animal welfare. So far, European legislation prescribes only documentation of stunning effectiveness in the Council Regulation (EC) No. 1099/2009. However, besides stunning, the day of slaughter involves a series of potential stressful elements for pigs (*Sus scrofa domesticus*) (Barton, 2004; Gregory, 2008; Støier et al., 2001), and at present no tool for documenting the welfare consequences of these is available.

The Welfare Quality® (WQ®) has developed a comprehensive protocol for assessment of welfare of pigs at abattoirs (Welfare Quality®, 2009). However, an aggregation model for welfare of finishing pigs on the day of slaughter is not yet available. For the welfare protocol proposed by WQ® for pigs on farms, the aggregation of welfare measurements was carried out in a hierarchical manner (Botreau et al., 2007). For simplicity and transparency reasons, a recent study on dairy cows presented an alternative welfare index based on the weighted linear sum of the prevalence of individual welfare measurements (Burow et al., 2013). Common to both models is the expert opinion underlying the weighting of the welfare measurements, a tool that has been used to assign weights for animal welfare measurements when overall welfare assessment is performed at herd level (Rousing et al., 2007; Jensen et al., 2012; Burow et al., 2013). In the present study, expert opinion was used to assign weights to animal welfare measurements on the day of slaughter.

One measurement of the stress responses of individual pigs exposed to acute stressors on the day of slaughter is changes in heart rate (Lewis et al., 2008; Correa et al., 2010; Correa et al., 2013). Pigs loaded using an electric prod had significantly higher heart rate during loading compared with pigs loaded using a compressed air prod or board and paddle (Correa et al., 2010). Thus, changes in heart rate may reflect the level of stress in pigs within single stages of the day of slaughter.
Consequently, in the present study, changes in heart rate were used to evaluate the developed animal welfare index (AWI).

Among the potentially stress-inducing stages on the day of slaughter for pigs are: the pick-up facilities, loading, transport, unloading, lairage and the race to the stunning chamber. Access to an assessment of welfare within the single stages on the day of slaughter as well as for the entire day of slaughter would provide a feedback mechanism for optimization of the welfare of the pigs. Furthermore, aggregation of the assessments into an index would allow simple comparison of the level of welfare between stages as well as between lorries, days, abattoirs etc. and can be used for marketing (Johnsen et al., 2001).

Hence, the aim of the present study was to develop an aggregated animal welfare assessment using expert opinion to establish the weights – an animal welfare index (AWI) – for finishing pigs on the entire day of slaughter, including an index within single stages as well as an overall index for the day of slaughter. To evaluate the developed AWI, a welfare assessment including a total of 45 pigs was carried out for each of the six stages and related to the average heart rate within stage. Our hypotheses were that different animal welfare measurements within stage, as well as the different stages from farm to slaughter, would be assigned different weights in an aggregated welfare assessment, and that the stage-AWI (AWI_{Stage}) and heart rate would follow a comparable pattern.

For the latter, we collected data for an animal welfare assessment as well as heart rate data in a ‘real life’ set up during which we collected data from pigs from five herds (see details in the material and methods’ section) from ‘farm to slaughter’. Not two farms are the same, as two transports or procedures at the abattoir nor are. For example, we included three herds from which pigs were moved to the pick-up pen the night before being transported to slaughter and two herds from which pigs were moved to the pick-up facility in the morning of the transport to slaughter. These two
situations cannot be compared directly. In real life set-ups, many factors are interacting, for that reason we cannot draw absolute conclusions, but the advantage is that the results are generalizable.

87 Materials and methods

88 Aggregation of measurements for an overall welfare assessment using expert opinion

89 An expert panel was selected to provide weights for selected welfare measurements for an aggregated assessment of welfare of finishing pigs on the day of slaughter by use of an online questionnaire (a copy of the questionnaire is available by contacting the corresponding author). In the recruitment, we focused on a narrow animal science expert panel using the following inclusion criteria: current employment in Denmark, expertise within health and welfare of finishing pigs via employment in the industry, authorities, private research centres or universities combined with an education within animal science, veterinary medicine or alike of at least the level of Master of Science. A list of 39 Danish experts was formed, and the selected experts were invited to participate. The invitation included information about the identity of other invited participants within the same organisation and suggested that the experts further invited supplementary candidates of relevance if possible. As a result, the questionnaire was completed by 24 of the 39 experts (response rate of 62 %) and by another 15 supplementary candidates suggested by experts from the original list. The respondents were employees from Danish abattoirs (two experts), universities (nine experts), the government authorities (three experts), production consultants (13 experts), employees from private research centres (eight experts), and other (four experts). The answers to the questionnaire were kept anonymous, but all respondents gave information on education and affiliation.

In the questionnaire, the experts were asked to assess the importance of selected welfare measurements (presented in Table 5 together with the results) on a 5-point scale, where 1 was “not
important” and 5 “essential” at six stages during the day of slaughter: in the pick-up facility, during loading, transport, unloading, lairage and in the race to the stunning chamber. In connection with questions about loading, unloading and the race to stunning, short video recordings were shown in order to establish a common basis for answering. Each expert was asked to put one answer to each measurement and invited to add further measurements, if essential measurements did not appear from the questionnaire. Eighteen of the experts added the following factors: availability of feed and water in the pick-up facilities, cover of pick-up facilities, non-slip surfaces, vocalizations (human and animal), noise, group size, temperature differences between in- and outdoor, bedding and ventilation on the lorry, pigs diagnosed as injured upon arrival at the abattoir, moving backwards or sideways in the race and a differentiation between a minor contact and a push by the automatic gate in the race. Additionally, the members of the expert panel were asked to score the importance of the different stages by the 5-point scale.

One expert (affiliation: other) assigned five “essential” for every measurement and was removed before further analyses. Thus, 38 expert opinions were included in the calculations of weights for the welfare index.

**Aggregation model**

The measurements were aggregated into an animal welfare index for each of the six stages of the day of slaughter ($\text{AWI}_{\text{Stage}}$). The $\text{AWI}_{\text{Stage}}$ was calculated relative to a theoretical maximum value within each stage and corrected for the different number of measurements per stage. The index can take on values from 0-100, and the higher the index the more severe the welfare consequences. The aggregation of the measurements was done using a weighted linear sum of prevalence on animal level, as described by Burow *et al.* (2013):

$$\text{AWI}_{\text{Stage}} = \sum_{i=1}^{x} P_i MW_i$$
where $P$ was the prevalence, and $MW$ was the weight determined by the expert panel of the individual measurement ($x_i$). Six stages were included, and the number of individual measurements within stages was: 1 (pick-up facility), 7 (loading), 2 (transport), 6 (unloading), 5 (lairage) and 4 (race to stunning).

The AWI\textsubscript{Overall} was calculated as the simple sum of the six stage indexes.

**Welfare assessment of pigs from farm to slaughter**

We examined whether the stage index and the average heart rate within each stage followed a comparable pattern throughout the day of slaughter. To do so, data describing animal welfare was collected from a total of 45 focal pigs from five commercial herds.

**The welfare assessment protocol**

The assessment protocol of the present study was based on the WQ® for finishing pigs at an abattoir. Supplementary to WQ®, we collected data from the pick-up pens, loading, transport as well as during unloading, lairage and race to the stunning chamber. During transport only outdoor temperature, duration of transport and heart rate of the pigs were recorded. The protocol included direct animal-based measurements such as slipping, falling, reluctance to move, turning back and skin damage. In addition to the WQ® measurements, we included the following measurements: duration of each stage, mixing with other pigs, postures of pigs during lairage, score of driving (pigs moved voluntarily or pigs moved by paddle or board), pigs being lifted by other pigs (overlapping), pigs moved by gate in the race and heart rate of pigs.

Our protocol for welfare assessment of finishing pigs on the day of slaughter addressed all four principles of the WQ® with some adjustments as described below. ‘Absence of prolonged hunger’ was indicated by hours of fasting. Only pigs staying at the abattoir for more than 12 hours are
required to be fed (Council Regulation (EC) No. 1099/2009), and since the pigs included in this study were only kept in lairage for approximately one hour, none of them were recorded as fasting during the study. To which extent the pigs had been fasting in the pick-up facilities was not recorded systematically and hence could not be included. We have not included measurements of ‘absence of prolonged thirst’ either, since the measurement ‘access to water’ as suggested by WQ® could not be recorded with the required precision under the given experimental conditions.

‘Comfort around resting’ was measured by the postures of the pigs. Bedding was provided on the lorries but neither in pick-up pens nor lairage pens since bedding is not required for stays of less than 12 hours (Council Regulation (EC) No. 1099/2009).

Lameness was assessed in the home pen by categorizing mobility as either 0: no lameness or 1: uneven walk. However, since no lame pigs were observed (0 pigs of the 45) this measurement was not included in the statistical analyses. The WQ® criterion ‘absence of disease’ addresses dead animals and lame animals with the score ‘not weight bearing’. These measurements were not included due to expected very low prevalence. At unloading at the abattoir, all pigs were inspected visually by a veterinary, and isolation/emergency procedures were available, but not considered relevant for the pigs within the present dataset. Pigs being moved by automatic gates in the race and pigs being lifted by other pigs in the race were included as measurements of the criterion ‘absence of pain induced by management procedures’. Sticking effectiveness was measured automatically using the VisStick® system in the abattoir (Borggaard et al., 2011).

‘Good human-animal relationship’ was assessed by scoring the handling of the pigs (driving). Vocalizations were discarded due to lack of possibility to distinguish focal pigs under commercial conditions. ‘Positive emotional state’ was measured by reluctance to move and turning back. ‘Expression of social behaviours’ and ‘expression of other behaviours’ are not included in the WQ® for assessment of pig welfare at abattoirs and were not included in the present protocol.
either. Our protocol is presented in Table 1, and the recordings of the measurements are described in detail in “Measurements”.

**Table 1.** Measurements included in the protocol for assessment of welfare of finishing pigs on the day of slaughter organized according to the principles and categories suggested by Welfare Quality®.

<table>
<thead>
<tr>
<th>Welfare principle</th>
<th>Welfare Criteria</th>
<th>Measurements</th>
<th>Recording</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good feeding</td>
<td>Absence of prolonged hunger</td>
<td>(Hours of fasting)</td>
<td>Pick-up pen</td>
</tr>
<tr>
<td></td>
<td>Absence of prolonged thirst</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Good housing</td>
<td>Comfort around resting</td>
<td>Posture</td>
<td>Lairage</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heart rate</td>
<td>From pick-up pen to sticking</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mixing</td>
<td>Pick-up pen, lairage</td>
</tr>
<tr>
<td></td>
<td>Thermal comfort</td>
<td>(Outdoor temperature)</td>
<td>During transport</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Durations</td>
<td>(Pick-up pen), loading, transport, breaks, unloading, lairage, race</td>
</tr>
<tr>
<td></td>
<td>Ease of movement</td>
<td>Slipping, falling</td>
<td>Loading, unloading, race</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Stocking density)</td>
<td>Pick-up pen, lairage</td>
</tr>
<tr>
<td>Good health</td>
<td>Absence of injuries</td>
<td>Lameness, skin damage</td>
<td>Pick-up pen and lairage</td>
</tr>
<tr>
<td></td>
<td>Absence of disease</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Absence of pain induced by management procedures</td>
<td>Overlapping, moved by automatic gate</td>
<td>Race</td>
</tr>
<tr>
<td>Appropriate behaviour</td>
<td>Expression of social behaviours</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Expression of other behaviours</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Good human-animal relationship</td>
<td>Driving</td>
<td>Loading, unloading</td>
</tr>
<tr>
<td></td>
<td>Positive emotional state</td>
<td>Reluctance to move, turning back</td>
<td>Loading, unloading</td>
</tr>
</tbody>
</table>

**Animals and herds**

This study was part of a larger project including 480 pigs from 12 commercial herds (Brandt et al., 2015). In the present study, a subsample of 45 focal pigs from five of the commercial herds was included. The total number of pigs delivered for slaughter from each herd was decided by the farmer and differed between herds (Table 2). A private Danish transport company selected pig producers who had registered at least 90 pigs to the relevant abattoir on the selected experimental
The pigs were transported in different commercial three deck Finkl lorries. The breed of the pigs was not recorded; however, most Danish slaughter pigs are crossbred Duroc (sire) and Landrace-Yorkshire (sow). The pigs were slaughtered at 100-110 kg of body weight corresponding to approximately six months of age.

**Data recordings during the day of slaughter**

The welfare was assessed observationally (Kjaer Ersboll *et al.*, 2004) on two Danish commercial abattoirs (slaughter rate: 820 pigs/hour using CO$_2$ group stunning and a slaughter line speed of 410 carcasses/hour) chosen as representative for abattoirs in Denmark. Data was collected in the spring of 2013. The 24-hour average outdoor temperature during data collection periods was 7 °C for abattoir A (17 pigs) and 15 °C for abattoir B (28 pigs). Since the data collection was based on commercial transports, already scheduled to pick up pigs at several sites per day, the duration of transport varied from 50 to 75 minutes (Table 2).

Data recordings were carried out at six stages during the day of slaughter: 1) in the pick-up facilities, 2) at loading, 3) during transport, 4) at unloading, 5) during lairage and 6) in the race to the stunning chamber.
Table 2. Duration of loading, transport, breaks during transport, unloading and holding time in lairage for the five herds included in the observational study of pig welfare on the day of slaughter.

The specified durations are rounded to the nearest 5 minutes.

<table>
<thead>
<tr>
<th>Herd</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of pigs/herd</td>
<td>146</td>
<td>90</td>
<td>215</td>
<td>146</td>
<td>215</td>
</tr>
<tr>
<td>No. of focal pigs</td>
<td>7</td>
<td>10</td>
<td>9</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>Loading, min</td>
<td>30</td>
<td>15</td>
<td>35</td>
<td>25</td>
<td>40</td>
</tr>
<tr>
<td>Transport, total, min</td>
<td>50</td>
<td>60</td>
<td>75</td>
<td>60</td>
<td>55</td>
</tr>
<tr>
<td>Break, total, min</td>
<td>-</td>
<td>50</td>
<td>-</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>Unloading¹, min</td>
<td>20</td>
<td>10²</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Lairage, min</td>
<td>45</td>
<td>90</td>
<td>95</td>
<td>90</td>
<td>95</td>
</tr>
<tr>
<td>Race³, min</td>
<td>15</td>
<td>15</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

¹Unloading: from first pig leaving the lorry until last pig had passed the veterinary inspection.
²Only the time taken to unload the 90 pigs in question.
³Duration of time spent in the last three compartments prior to stunning.

Procedures

The day before slaughter, the focal pigs were spray marked for individual recognition. Marking was done by trained technicians while the pigs were either in the home pen or in the pick-up facility. In addition, the focal pigs were tattooed on the hindquarters for slaughter line identification.

Data collection was intentionally performed under commercial conditions, which is why no instructions regarding handling of the pigs were given to farmers or staff.

The pigs were moved from their home pens to conventional pick-up facilities by the farmers either on the day before slaughter (three herds) or in the morning before slaughter (two herds). The pigs were mixed at this point. The pigs were loaded onto a commercial lorry by the driver following normal Danish commercial procedures.

On arrival at the abattoir, all pigs from a lorry were unloaded according to the procedures of the abattoir and moved by the abattoir staff to lairage where the focal pigs were allocated to six
adjoining pens (pen area: 8.6 m², height of solid walls: 1.06 m, pigs in adjoining pens), each holding 15 pigs.

Lairage duration aimed at 60 minutes for assessment of undisturbed behaviour and subsequently 30 minutes for skin damage assessment, but depended on the need for pigs at the slaughter line (85 min ± 23 min; Table 2). After lairage, the pigs were driven by abattoir staff using commercial rattle sticks from the lairage pens into a race with automatic push-hoist gates (Automatic Driveway Systems, Butina A/S, Holbaek, Denmark at abattoir A; SFK Systems A/S, Kolding, Denmark at abattoir B). The gates moved the pigs towards and into the stunning chamber. The pigs were stunned in groups of seven to eight in 90 % CO₂ for 3 minutes (Backloader, Butina A/S, Holbaek, Denmark), shackled and stuck according to legislation (Council Regulation (EC) No. 1099/2009).

Measurements
The handling and behaviour of the pigs during loading, unloading and in the race to the stunning chamber was video recorded (HERO2, GoPro, © 2013 Woodman Labs, Inc.). At loading, one camera was secured to the lorry roof, and during unloading one camera was placed at the abattoir door and one just above the veterinary inspection. In the race to the stunning chamber, a camera was attached to the gates of each of the last three compartments. One trained observer performed all video analyses. During lairage, the behaviour of the pigs was observed by direct observation.

Handling during loading and unloading
Scoring of the handling and pig behaviour was done during loading and unloading. The handling of the animals during loading and unloading was recorded by focal animal one-zero sampling (Martin and Bateson, 2007) and a scale ranging from 0 to 3, in which 0 = the pig moved voluntarily or was driven along by the herd, 1 = the staff used a rattle stick or board (just touching), 2 = the staff used a
rattle stick, board or hand (single stroke), and 3 = the staff used a rattle stick, board or hand (repetitive strokes). For each pig, the highest score was used for further analysis. At unloading, the recordings were initiated at the ramp of the lorry and terminated when the pigs had passed a visual veterinary inspection just before entering the lairage area.

Handling in the race

In the race to the stunning chamber, it was recorded whether the pigs were physically moved by the automatic gates (the pig was pushed forward by the automatic gate) or were overlapping (Correa et al., 2010) other pigs due to lack of space (Table 3). The recordings were performed continuously counting the number of events at focal pig level.

Behavioural recordings

The present ethogram was based on a previous study (Brandt et al., 2013) (Table 3). At loading and unloading, the behavioural recordings included the frequency of reluctance to move, turning back, slipping and falling.
Ethogram of pig behaviour used for welfare assessment of finishing pigs during the day of slaughter obtained from video recordings at loading, unloading and in the race to the stunner at an abattoir. During lairage, the behavioural observations were obtained by direct observation.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Behaviour</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>l, u, r</td>
<td>Slipping</td>
<td>The pig slips at least one leg, the body does not come into contact with the floor (Dalmau et al., 2009).</td>
</tr>
<tr>
<td>l, u, r</td>
<td>Falling</td>
<td>The pig loses balance on one or more legs, and falls to its knees, or another part of the body other than legs comes into contact with the floor (Dalmau et al., 2009).</td>
</tr>
<tr>
<td>l, u</td>
<td>Reluctance to move when being driven</td>
<td>The pig stops for &gt; 2 sec (Correa et al., 2010; Dalmau et al., 2009; Welfare Quality®, 2009) when being driven forward by rattle stick or board. When the pig resumes forward movement, the event is ended, and a new event recorded when the pig stops again for &gt; 2 sec.</td>
</tr>
<tr>
<td>l, u</td>
<td>Turning back when being driven</td>
<td>The pig turns more than 90 degrees away from the direction of movement when being driven forward by rattle stick or board (Welfare Quality®, 2009).</td>
</tr>
<tr>
<td>la</td>
<td>Posture during lairage</td>
<td>Lying: the body of the pig is in contact with the floor, either sternally or laterally. Sitting: the pig rests on its thighs and front legs (Terlouw et al., 2009). Standing: the pig is supported on all four legs, active or inactive.</td>
</tr>
<tr>
<td>l, u, r</td>
<td>Overlapping</td>
<td>During driving, the pig was lifted by another pig, while its forelegs are placed on the back of another pig (Correa et al., 2010).</td>
</tr>
</tbody>
</table>

1 = loading, u = unloading, la = lairage, r = race to stunning

Behavioural observations during lairage were conducted using direct observation and instantaneous sampling (Martin and Bateson, 2007) by three observers simultaneously. For a total of 60 minutes, each observer recorded pig behaviour in two adjoining pens during observation periods of two minutes. At the initiation of each observation period, the posture of the focal pigs was recorded (standing, sitting or lying).
In the last three compartments prior to the stunning chamber, the behavioural recordings of the focal pigs included the occurrence of slipping and falling recorded by one-zero sampling (Martin and Bateson, 2007).

**Clinical recordings**

Skin damage was recorded twice: in the pick-up facilities prior to loading and after one hour of lairage. Skin damage was assessed on the head/ear, the shoulder, the middle and the hindquarters, separately, using a four-point scale (Aaslyng et al., 2013): 0: None or minor scratches, 1: few (< 3) short (up to 3 cm) scratches, 2: > 3 short or ≥ 1 long scratch(es) (> 3 cm), 3: ≥ 1 wound(s). A total skin damage score was calculated per pig as the sum of the individual scores. One experienced observer performed all assessments.

**Physiological recordings**

Heart rate was recorded every second using the Polar Team2 Pro equipment (Polar, Helsinki, Finland). Heart rate belts were fitted around the chest of the pigs while in the pick-up facility approximately one hour prior to loading, by use of a special designed nylon strap. Water and gel were applied to the sensors to improve connection. The belts were removed after sticking. Subsequent error correction was performed automatically by the Polar Team2 Pro software with the settings “correction efficiency” = moderate and “correction threshold” = 6 bpm. The Polar system started a new file, when the signal went missing. Files of less than five minutes were not included, and values below 60 bpm were removed before analyses.
Preparation of data for statistical analyses

All AWI measurements were categorized on a three-point scale (0, 1, 2). In case of missing values, the average of the measurements concerned was used. For duration of transport, breaks during transport, duration of lairage and latency to lie down, categorization was literature based. The limits for duration of transport were based on Gade et al. (2007) showing increased mortality for transports exceeding 200 km (corresponding to approximately three hours) compared to shorter transports. Breaks during transport may affect animal welfare negatively (Gosálvez et al. 2006), and limits were determined similar to the duration of loading. Limits for duration of lairage were set by the WQ®.

After 15 minutes of lairage, up to 80% of the finishing pigs already lay down (Brandt et al., 2013), and we categorized latency to lie down in intervals of 15 minutes. Categorization of percentage of time lying down was based on the 60 minutes target duration, allowing pigs to stand and/or sit for up to 30 minutes by assigning score 0, however, in other cases, when lairage duration may be increased compared to the 60 minutes, these limits need reconsideration.

No difference was observed in the mixing procedures between the five herds included in the study, which is why mixing was not included in the present welfare index. For stocking density (0.65 m²/pig (Council Directive 2001/88/EC)), fasting (up to 12 hours of fasting at the abattoir) and duration of the stay in the pick-up facility none of the five herds exceeded the recommendations. In these cases, compliance with the recommendations was categorized as 0, and hence, all 45 pigs received a score of 0, and the measurements were not included in the index.

For the other measurements, the categorization was based on mathematical subdivision of output. For the measurements reluctance to move, turning back, slipping, falling, overlapping and moved by gate, a score of 0 corresponded to no occurrences, 1 to 1 occurrence and 2 to 2 or more occurrences. In case of a definite maximum, the score was split into three equal parts. Thus, skin
damage (maximum score of 12) was divided into score 0: 0-4, 1: 5-8 and 2: 9-12. In case of an indefinite maximum, the authors defined the limits. The negative aspects of loading and unloading have been accounted for by including recordings of slipping, falling, reluctance to move, turning back and driving score. Hence, a short duration of loading and unloading is considered positive and categorized as such. The categorizations can be seen in Table 4. Driving style, temperature, aggression and mounting behaviour were not included in the index in the present study.

**Table 4.** Suggested measurements for welfare assessment of finishing pigs during the day of slaughter categorized on a three-point scale (0, 1, 2).

<table>
<thead>
<tr>
<th>Skin damage, score</th>
<th>Duration loading, min</th>
<th>Duration transport, hours</th>
<th>Duration of breaks during transport, min</th>
<th>Duration unloading, min</th>
<th>Duration lairage, hours</th>
<th>Latency to lie down, min</th>
<th>Time lying down, %</th>
<th>No. of postural changes</th>
<th>Driving style, score</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0-4</td>
<td>0-1</td>
<td>0-29</td>
<td>0-14</td>
<td>0-14</td>
<td>100-50</td>
<td>0-4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>5-8</td>
<td>1-3</td>
<td>30-59</td>
<td>15-29</td>
<td>15-29</td>
<td>49-25</td>
<td>5-9</td>
<td>1-2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>9-12</td>
<td>≥60</td>
<td>≥30</td>
<td>≥12</td>
<td>≥30</td>
<td>24-0</td>
<td>≥10</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

**Statistical analyses**

The relationship between the expert scores and expert affiliation was analysed using a non-parametric one-way analysis of variance (Kruskal-Wallis test). The different affiliations were represented by a relatively few experts, and the number of experts from each affiliation differed. Each stage was described by up to nine welfare measurements, which are not comparable between stages. Therefore, the analysis was run for each combination of stage and welfare measurement.

The significance level used was \( P < 0.05 \), but the p-values in the pairwise comparison were adjusted subsequently using Bonferroni correction in order to account for multiple comparisons. Subsequently, differences in expert scores of the individual welfare measurements were tested within stage. The distributions were not all approximate normal distributions, and the entire scale was not used every time, therefore a non-parametric one-way analysis of variance (Kruskal-Wallis test) was used.
test) was used. The relationship between stage scores was analysed using $\chi^2$-test. The non-parametric tests were carried out using SAS (Copyright (c) 2002-2008 by SAS Institute Inc., Cary, NC, USA, All Rights Reserved).

The relationship between stage and average heart rate within stage was analysed using a linear mixed model:

$$ Y_{ij} = \mu + s_i + P_j + e_{ij} $$

where $Y_{ij}$ was average heart rate within stage, stage ($s$) was included as fixed effect and pig ($P$) ($j = 1, 2, 3, ..., 45$) was included as a random effect $Pig_j \sim N(0, \sigma^2_S)$. $e_{ij}$ was assumed to be independent and normally distributed, which was confirmed by graphic representation (not shown) and tests (e.g. Shapiro-Wilk and Kolmogorov-Smirnov) using SAS.

The correlation between average expert stage scores and average heart rate per stage was calculated using Spearman correlations. Similarly, the correlation between heart rate and AWI$_{Stage}$ was calculated.

The linear mixed model and the Spearman correlations were analysed using R (R Core Team, 2014) and, in particular, the extension package “lmerTest” (Kuznetsova et al., 2014).

**Results**

**Expert opinion**

The affiliations were split on experts from the Danish abattoirs (two experts), universities (nine experts), the authorities (three experts), production consultants (13 experts), employees from private research centres (eight experts), and other (three experts). No significant effects of expert affiliation on weighting of the scores were found, and the mean scores of the 38 experts were therefore used for further calculations. Subsequently, we tested for differences in the scores assigned by the experts for each individual welfare measurement within stage and found significant differences in
all stages ($P < 0.05$). Therefore, the welfare measurements need to be weighted in an aggregated animal welfare index.

Table 5 presents the means and the weights of the individual measurements within each stage. The weights were calculated by setting the minimum value to 1 within stage and calculate the weights of the other measurements relative to the minimum value. The AWI$_{\text{Stage}}$ was then calculated by multiplying the frequency with the weight.
Table 5. Expert opinion was used to assign weights for selected welfare measurements in order to assess the welfare of finishing pigs on the day of slaughter. The expert panel scored the importance of different measurements within each stage of the day of slaughter. Weights were calculated based on the score means. The measurements were listed with the highest score first. The measurements in parenthesis were not included in the Animal Welfare Index (AWI) calculations.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Measurement</th>
<th>Mean</th>
<th>S.D.</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pick-up pen</td>
<td>(Mixing)</td>
<td>4.1</td>
<td>0.9</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>Skin damage</td>
<td>3.9</td>
<td>0.9</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>(Stocking density)</td>
<td>3.9</td>
<td>0.8</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>(Duration)</td>
<td>3.5</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>(Fast)</td>
<td>3.2</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Loading</td>
<td>Driving</td>
<td>4.3</td>
<td>1.0</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>Falling</td>
<td>3.8</td>
<td>1.1</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>Overlap</td>
<td>3.2</td>
<td>1.1</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>Slipping</td>
<td>3.1</td>
<td>1.1</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>Duration</td>
<td>2.9</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>Turning back</td>
<td>2.7</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>Reluctance to move</td>
<td>2.5</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Transport</td>
<td>(Temperature)</td>
<td>4.6</td>
<td>0.6</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>(Driving style)</td>
<td>3.7</td>
<td>0.9</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>Duration of transport</td>
<td>3.5</td>
<td>1.3</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>Duration of breaks</td>
<td>3.3</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Unloading</td>
<td>Driving</td>
<td>4.3</td>
<td>1.0</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>Falling</td>
<td>3.8</td>
<td>1.1</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>Slipping</td>
<td>3.2</td>
<td>1.0</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>Turning back</td>
<td>2.9</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>Duration</td>
<td>2.8</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>Reluctance to move</td>
<td>2.6</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Lairage</td>
<td>(Mixing)</td>
<td>4.2</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>Skin damage</td>
<td>4.0</td>
<td>0.9</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>(Aggression)</td>
<td>3.9</td>
<td>0.9</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>Duration</td>
<td>3.5</td>
<td>1.1</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>(Being mounted)</td>
<td>3.3</td>
<td>1.0</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>Latency to lie down</td>
<td>3.2</td>
<td>1.0</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>% of time lying down</td>
<td>3.2</td>
<td>1.0</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>(Mounting)</td>
<td>2.9</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>No. of posture changes</td>
<td>2.8</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Race to stunner</td>
<td>Falling</td>
<td>4.0</td>
<td>1.0</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>Overlapping</td>
<td>3.5</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>Moved by gate</td>
<td>3.5</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>Slipping</td>
<td>3.3</td>
<td>0.9</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Subsequently, we tested for differences in the stage scores assigned by the experts and found no significant differences. Loading, unloading and lairage were numerically scored higher than pick-up, transport and race (Table 6).

Table 6. An expert panel scored the importance of different stages of the day of slaughter in finishing pigs on a five-point scale. Means are reported.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Mean</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pick-up pen</td>
<td>3.8</td>
<td>1.0</td>
</tr>
<tr>
<td>Loading</td>
<td>4.3</td>
<td>0.8</td>
</tr>
<tr>
<td>Transport</td>
<td>3.7</td>
<td>0.9</td>
</tr>
<tr>
<td>Unloading</td>
<td>4.0</td>
<td>0.8</td>
</tr>
<tr>
<td>Lairage</td>
<td>4.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Race</td>
<td>3.9</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Frequencies of behavioural responses and clinical observations

The frequencies of the behavioural responses when the pigs were moved (either manually or automatically) are shown in Table 7.
Table 7. Finishing pigs’ behaviour observed on the day of slaughter. The frequencies of the observed behavioural responses during loading, unloading and in the race to the stunner are expressed as percentage of pigs for whom the behaviour occurred, with no regard to the frequency per pig. Not all of the 45 pigs were retrievable from the video recordings.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Loading</th>
<th>Unloading</th>
<th>Race</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slipping</td>
<td>0</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Falling</td>
<td>3</td>
<td>3</td>
<td>21</td>
</tr>
<tr>
<td>Reluctance to move</td>
<td>6</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Turning back</td>
<td>6</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Driving</td>
<td>63</td>
<td>16</td>
<td>-</td>
</tr>
<tr>
<td>Overlapping</td>
<td>11</td>
<td>-</td>
<td>12</td>
</tr>
<tr>
<td>Moved by gate</td>
<td>-</td>
<td>-</td>
<td>45</td>
</tr>
</tbody>
</table>

In lairage, the average latency to lie down was 8.2 min (± 7.6 min), the average percentage of time spent lying down was 71 % (± 23 %) and the average number of postural changes was 4.0 (± 2.6) (from lying down to either sitting or standing, changes from sitting to standing and standing to sitting are not considered).

Skin damage score (n = 35) assessed on hindquarters, middle, shoulder and head/ear was on average 0.4, 0.3, 0.9 and 0.7, respectively, for pick-up pen and 0.3, 0.2, 0.9 and 0.7, respectively, after one hour in lairage. The level of skin damage was approximately the same after lairage as in the pick-up pen, indicating a low incidence of fighting.

Heart rate

The average heart rates per stage are shown in Table 8. The average heart rate was significantly higher during loading, unloading and race compared with the pick-up pen, transport and lairage. There was a significant difference between pigs ($P < 0.00001$).
Table 8. Heart rate was recorded for finishing pigs from the pick-up pen until sticking. The average heart rate for each stage was calculated per pig. LS means of all pigs per stage are reported together with the minimum and maximum value. Different letters indicate a significant difference at $P < 0.05$.

<table>
<thead>
<tr>
<th>Stage</th>
<th>HR, LS means</th>
<th>S.E.</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pick-up pen (n=45)</td>
<td>123&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.6</td>
<td>87</td>
<td>168</td>
</tr>
<tr>
<td>Loading (n=45)</td>
<td>139&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.6</td>
<td>97</td>
<td>190</td>
</tr>
<tr>
<td>Transport (n=45)</td>
<td>120&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.6</td>
<td>100</td>
<td>154</td>
</tr>
<tr>
<td>Unloading (n=45)</td>
<td>132&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.6</td>
<td>97</td>
<td>170</td>
</tr>
<tr>
<td>Lairage (n=45)</td>
<td>114&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.6</td>
<td>94</td>
<td>160</td>
</tr>
<tr>
<td>Race (n=44)</td>
<td>134&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>2.6</td>
<td>83</td>
<td>179</td>
</tr>
</tbody>
</table>

Relationship between the AWI<sub>Stage</sub> and heart rate

The average Animal Welfare Index (AWI) per stage is illustrated in Figure 1. Loading and lairage received the largest average AWI while transport received the lowest.
Figure 1. The Animal Welfare Index (AWI) obtained from 45 finishing pigs on the day of slaughter for each of the six stages. Loading and lairage received the largest average AWI.

There was no relationship between the score per stage assigned by the expert panel (as seen in Table 6) and the average heart rate within stage ($r = 0.61, P = 0.19$), and no significant correlations between AWI\textsubscript{Stage} and average heart rate per stage ($r = 0.43, P = 0.40$) were found (Figure 2). This was especially due to a high AWI\textsubscript{Lairage} having a low heart rate. If lairage was removed, the correlations were ($r = 0.95, P = 0.01$) and ($r = 0.84, P = 0.08$), respectively.
Figure 2. Average AWI for each stage against heart rate for 45 finishing pigs. There was no significant correlation between heart rate and the AWI ($r = 0.43, P = 0.40$). Stages where the pigs are being moved from one place to another (loading, unloading and race) had a higher heart rate, but not necessarily an increased AWI. Removing lairage resulted in an increased correlation ($r = 0.84, P = 0.08$).

The average AWI_{Overall} was 54.3 (min = 22.0; max = 84.1; S.D. = 14.9). None of the pigs had an index of 0 for all stages of the day of slaughter. The theoretical maximum (the prevalence of all measurements equalled 100 %) was 600. Relative to a theoretical maximum for AWI_{Overall} the average AWI_{Overall} was 9.0 (min = 2.5; max = 14.0; S.D. = 2.5).

Discussion

In this study, we developed an animal welfare index (AWI) for finishing pigs on the day of slaughter based on principles and criteria of WQ® for finishing pigs at an abattoir. We added a number of measurements and included assessment in the pick-up facility, during loading at the farm and during transport. Hence, the overall index included 25 measurements. Expert opinion was used.
to provide weights for aggregating measurements within each stage, based on which the animal welfare index was calculated as a weighted linear sum of prevalence of measurements on animal level. The welfare measurements attained different weights within each stage, but no differences between stage weights were found. The weights assigned by the expert panel for the individual stages were correlated to pig heart rate when lairage was excluded, and tended to correlate between heart rate and an AWI<sub>Stage</sub>. In conclusion, a welfare index within single stages may be useful for intra-stage comparison between days, abattoirs etc., but will be less useful for comparison between stages.

**Expert opinion**

Expert opinion was used to calculate the weights for the single measurements. Expert opinion is a commonly used method to provide weights for individual measurements when performing overall assessments of animal welfare (Rousing <em>et al.</em>, 2007; Welfare Quality®, 2009; Jensen <em>et al.</em>, 2012; Burow <em>et al.</em>, 2013). However, one disadvantage of this approach is the possible dependency of the particular panel. At present, no golden standards exist of the number of experts to include in a panel, or their affiliations or distribution among different stakeholders, but panels including 8/10 experts (<em>Jensen et al.</em>, 2012), 13 (<em>Rodenburg et al.</em>, 2008) and 24 experts (Lievaart and Noordhuizen, 2011) have been reported from studies of animal welfare.

In the WQ®, when aggregation of measurements into an index was developed, a panel of a relatively few animal welfare science experts was included (Bonde <em>et al.</em>, 2009). At a later stage when herd welfare categories were developed, experts covered different stakeholder groups (<em>Veissier et al.</em>, 2011) as different stakeholders e.g. producers and non-producers may have different views on animal welfare (Sørensen and Fraser, 2010; Veissier <em>et al.</em>, 2011). We decided, in this study, to use a panel of experts all having at least a master degree in animal science, since we aimed
at scientific expert opinion of welfare to calculate weights to enter into the index and not to investigate the different opinions within different groups of stakeholders. Thus, the present aggregation of measurements was similar to the first step of the WQ®, and we applied similar principles for the selection of experts. However, to increase the reliability of the results, a relatively high number of experts were included (n=38) compared with the WQ® (n=5) (Botreau et al., 2008). Different expert affiliations were included in the present study since the experts may in general be biased of the field in which they primarily work as suggested by Botreau et al. (2007) in order to consider different affiliations. No significant effects of expert affiliation on weighting of the individual welfare scores were found, but the measurements received different scores within stage. Hence, the weights presented in this study were calculated as a mean based on the panel of 38 experts.

**Animal welfare index (AWI)**

If the Animal Welfare Index (AWI) is highly correlated to measurements that can be analysed automatically, e.g. lactate, this would provide a tool for systematic documentation of animal welfare. It is therefore necessary to aggregate the individual welfare measurements to an index instead of only reporting the single measurements. For transparency reasons (as explained further by Burow et al., 2013) we decided to use the weighted linear sum of prevalence and not the hierarchical model as reported by the WQ (Welfare Quality®, 2009). In WQ®, the welfare measurements were initially transformed into scores in order to reflect compliance with the 12 welfare criteria, according to which the single scores were combined to form principle scores, and finally an overall assessment was made (Veissier et al., 2011). In the present study, an index was calculated for each pig, and the average AWI_{Stage} was used to illustrate a level of welfare between stages, as shown in Figure 1. Within each stage, the same
index level can result from different welfare measurements, which is why experts were asked to determine the importance of the different measurements. The use of the linear sum of prevalence means that if a pig is e.g. slipping and falling, the aggregated welfare assessment is the sum of those two measurements – neither more nor less. In contrast, WQ® found that their expert panel did not follow a linear reasoning (Veissier et al., 2011).

In the present study, each measurement was transformed into a score of 0, 1 or 2, the definitions of which have a large influence on the welfare index. Alternatively, data could have been categorized into 0/1 assigning 0 to values below data average and 1 to values above data average as suggested by Knage-Rasmussen et al. (2014). However, information will be lost when using only two scores, and this approach would be based on the average incidence when defining levels of acceptability (score 0) and non-acceptability (score 1), irrespectively of the average level. Burow et al. (2013) included graded (0/1/2) and non-graded (0/2) measurements in their model. Further, expert opinion was used to assign weights to score 2 in relation to score 1 for the graded measurements (Burow et al., 2013). Phythian et al. (2011) used expert opinion to select welfare indicators for welfare assessment, and in WQ®, expert opinion was used to assign weights for graded measurements (Veissier et al., 2011). Expert panel opinion can be used to decide on the limits, however, if filling in the questionnaire becomes too time consuming and complicated, the respondent percentage may decrease and thereby also the generalizability. In the present study, we decided to keep the questionnaire as short and simple as possible aiming at a high number of respondents, perhaps at the expense that we have decided the limits based on literature showing the influence of the different factors on welfare, thereby potentially undermining the expert weights for single measurements.
Heart rate and AWI

No significant relationship between the heart rate per stage and the expert panel stage scores or the average AWI_{Stage} was found. Stages during which the pigs were moved from one point to another (loading, unloading and race) had an increased heart rate compared with stages during which the pigs were kept in one place (pick-up, transport and lairage), but not necessarily an increased AWI_{Stage} (Figure 2). Heart rate has previously been used as a measurement for acute stress in pigs. Similarly to the present results, average heart rate was increased during loading and unloading in previous studies (Correa et al., 2010; Correa et al., 2013). In addition, other acute stressors such as exposure to a novel environment and handling (Lewis et al., 2008) as well as the use of an electric prod (Correa et al., 2010) may lead to increased heart rate in pigs. The lack of correlation between heart rate and stage scores and AWI_{Stage} needs further examination in a larger dataset involving more extremes. However, the animal welfare index is not directly a measurement of an acute stress response, which may explain the lack of correlations. Especially for some stages, such as lairage, the lack of correlation with heart rate was expected. This may be caused by the relatively long duration of the stay in the lairage pens, since an indicator of sympathetic activation such as heart rate might not be appropriate to reflect the experience of the pigs during long periods. The relatively high AWI found for lairage may in part be due to the invariable order of the stages on the day of slaughter, during which lairage was one of the last stages experienced by the pigs, and they consequently may have responded to carry-over effects from previous stages. Hence, comparison of the level of welfare expressed by an animal welfare index between different stages of the day of slaughter (or any other multistage event) is not straightforward. As mentioned, several factors differed between the stages as during e.g. loading, unloading and in the race, the pigs were being moved from one place to another (either manually or automatically) whereas in the pick-up facility, during transport and in lairage, the pigs were kept in
one place and had the opportunity to rest, however, depending on the behaviour of their pen mates. We sought to accommodate these differences by including different welfare measurements at the different stages, but taken together the combination of the differences between stages and measurements within stage as well as their invariable order complicated the comparison of the level of welfare expressed by an AWI$\text{Stage}$. Thus, based on these arguments, we suggest that a welfare index within single stages may be useful for intra-stage comparison between days, abattoirs etc., but will be less useful for comparison between stages.

Dalmau et al. (2009) compared single measurements from the WQ® between 10 abattoirs in Spain and concluded that the assessment can be used to discriminate between abattoirs. Although they never attempted to aggregate the measurements into an overall welfare assessment, the difference between abattoirs would likely be evident also in an overall welfare score, which would make a comparison of multiple abattoirs foreseeable. We therefore developed an overall index using expert opinion, which can be used to compare levels of welfare between different lorries or abattoirs and also to correlate to various post mortem measurements. In the future, systematic documentation of welfare within the stages from farm to slaughter in commercial abattoirs as part of daily routines may be used as a tool to improve the level of welfare by a feed-back mechanism, i.e. if the monitored measurement of welfare suddenly increases above a specified threshold, an alarm could inform the abattoir staff for them to locate and correct the cause for the subsequent batch of pigs.

**Conclusion**

The present study has led to the development of a welfare assessment protocol for finishing pigs from farm to slaughter based on the WQ® principles. Expert opinion was used to assign weights for individual welfare measurements as well as individual stage weights, based on which a model for animal welfare assessment within stages (AWI$\text{Stage}$) and across stages (AWI$\text{Overall}$) was calculated.
The underlying complexity of the AWI means that especially the AWI\textsubscript{Stage} can be used to compare the level of welfare between different herds, lorries, abattoirs etc. The welfare measurements attained different weights within individual stages, but there was no difference between stage weights. Within stage of the day of slaughter, heart rate correlated with expert stage weights and tended to correlate with AWI\textsubscript{Stage}, when lairage was excluded. Thus, a welfare index within single stages may be useful for intra-stage comparison between days, abattoirs etc., but will be less useful for comparison between stages. Future studies should focus on possible correlations between AWIs and relevant post mortem measurements, which by use of technological solutions may be implemented at abattoirs and used in daily operations as on-site tools for continuous monitoring of welfare of finishing pigs to document the level of welfare on the day of slaughter.

Animal welfare implications

The day of slaughter necessitates acute stressors such as moving and mixing with unfamiliar pigs, which potentially affect animal welfare negatively. This study has developed an animal welfare index for the entire day of slaughter as well as for single stages, of which especially the AWI\textsubscript{Stage} may be used to compare the level of welfare between different herds, lorries, abattoirs etc. and which may form the basis of future examinations of possible on-site tools to quantify the level of welfare of finishing pigs on the day of slaughter.

Acknowledgements

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the Pig Levy Fund for funding the project.
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Revision and supplemental to PaperIII

Edwards et al. (2010a) studied lactate in whole blood and not in plasma as written in the introduction in PaperIII.

Table 4, 6 and 7 including S.D. values are shown below. Correlation coefficients in Table 6 were recalculated using Spearman correlations.

Table 4. At exsanguination, blood was collected from the finishing pigs and analysed for plasma concentration of lactate, glucose, t-protein, albumin and creatine kinase activity. In addition, the blood temperature was measured. Forty-five minutes after slaughter, the pH (pH45) was measured. The average, S.D. and range of each measurement as well as the number of pigs involved are presented.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Average</th>
<th>S.D.</th>
<th>min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate, mM</td>
<td>418</td>
<td>10.3</td>
<td>3.1</td>
<td>3.8</td>
<td>29.3</td>
</tr>
<tr>
<td>CK, U/L</td>
<td>418</td>
<td>7,800</td>
<td>11,000</td>
<td>600</td>
<td>120,000</td>
</tr>
<tr>
<td>T-protein, g/L</td>
<td>418</td>
<td>70.7</td>
<td>5.1</td>
<td>57.8</td>
<td>88.6</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>418</td>
<td>38.9</td>
<td>3.0</td>
<td>29.0</td>
<td>47.1</td>
</tr>
<tr>
<td>Glucose, mM</td>
<td>418</td>
<td>15.0</td>
<td>5.1</td>
<td>3.6</td>
<td>31.5</td>
</tr>
<tr>
<td>Blood temp, °C</td>
<td>262</td>
<td>38.8</td>
<td>0.7</td>
<td>36.0</td>
<td>40.0</td>
</tr>
<tr>
<td>pH45</td>
<td>276</td>
<td>6.6</td>
<td>0.2</td>
<td>5.9</td>
<td>7.3</td>
</tr>
</tbody>
</table>

Table 6. Skin damage scores of finishing pigs, obtained in the home pen, in the pick-up pen, after one hour in lairage and post-mortem using a four-point scale. The theoretical maximum for post-mortem assessment was 9 (as opposed to 12 for the live assessments).

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Skin damage score, mean (min - max)</th>
<th>S.D.</th>
<th>Correlation (Spearman) with logck</th>
</tr>
</thead>
<tbody>
<tr>
<td>Home pen</td>
<td>470</td>
<td>1.0 (0 - 5)</td>
<td>1.1</td>
<td>0, NS (n=411)</td>
</tr>
<tr>
<td>Pick-up pen</td>
<td>357</td>
<td>1.7 (0 - 7)</td>
<td>1.5</td>
<td>0.31, P &lt; 0.000001 (n=327)</td>
</tr>
<tr>
<td>After lairage</td>
<td>413</td>
<td>1.9 (0 - 6)</td>
<td>1.4</td>
<td>0.41, P &lt; 0.000001 (n=377)</td>
</tr>
<tr>
<td>Post-mortem</td>
<td>398</td>
<td>1.5 (0 - 6)</td>
<td>1.4</td>
<td>0.39, P &lt; 0.000001 (n=361)</td>
</tr>
</tbody>
</table>

Table 7. The relative AWI for each stage and the relative AWIOverall for finishing pigs on the day of slaughter. The average and range of each AWI on a 0-100 scale and the number of pigs involved are presented.

<table>
<thead>
<tr>
<th>AWIStage</th>
<th>n</th>
<th>Mean</th>
<th>S.D.</th>
<th>min</th>
<th>max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pick-up</td>
<td>317</td>
<td>9.9</td>
<td>4.6</td>
<td>0</td>
<td>21.1</td>
</tr>
<tr>
<td>Loading</td>
<td>357</td>
<td>8.7</td>
<td>7.4</td>
<td>0</td>
<td>47.1</td>
</tr>
<tr>
<td>Transport</td>
<td>360</td>
<td>1.5</td>
<td>2.8</td>
<td>0</td>
<td>6.8</td>
</tr>
<tr>
<td>Unloading</td>
<td>402</td>
<td>8.6</td>
<td>7.3</td>
<td>0</td>
<td>45.6</td>
</tr>
<tr>
<td>Lairage</td>
<td>299</td>
<td>16.9</td>
<td>11.6</td>
<td>0</td>
<td>56.4</td>
</tr>
<tr>
<td>Race</td>
<td>367</td>
<td>7.9</td>
<td>8.7</td>
<td>0</td>
<td>36.4</td>
</tr>
<tr>
<td>Overall</td>
<td>103</td>
<td>39.1</td>
<td>10.2</td>
<td>18.4</td>
<td>62.1</td>
</tr>
</tbody>
</table>

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5.3 PaperIII: The relationship between selected physiological post-mortem measures and an overall pig welfare assessment from farm to slaughter. Published in Livestock Science 180 (2015) 194-202

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The relationship between selected physiological post-mortem measures and an overall pig welfare assessment from farm to slaughter

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Day of slaughter

A B S T R A C T

On the day of slaughter, stressors associated with the handling, transport and lairage of pigs may have a significant impact on animal welfare. A welfare assessment based on the Welfare Quality® (WQ®) principles involving 480 pigs from 12 different commercial herds and including behavioural, clinical and physiological measures was carried out from farm to slaughter with the aim of investigating the relationship between selected post-mortem physiological measures and an aggregated animal welfare index (AWI). Blood temperature was quantified at exsanguination, and a blood sample collected at exsanguination was analysed for plasma concentration of glucose, lactate, albumin, total protein and creatine kinase activity. pH was measured 45 min after sticking in the m. longissimus dorsi (LD).

All welfare measures were categorised on a three-point scale (mild level: 0, moderate level: 1, severe level: 2) and aggregated into an AWI using a weighted (based on expert opinion of 38 experts) linear sum of prevalence on animal level. An overall AWI (AWIOverall) and an AWI per stage (AWIStage) were calculated. For AWIUnloading, significant relationships with the plasma concentration of glucose (positive), creatine kinase activity (positive) and total protein (negative) content of the blood were found. Furthermore, significant positive relationships between AWIRace and the plasma concentration of lactate and albumin were found. Based on the current data, we suggest one method to identify AWIs above vs. below a certain threshold using the post-mortem measures with AWIRace and lactate as an example.

In conclusion, physiological post-mortem measures may, after the development of technological solutions for implementation at abattoirs, be used in daily operations as on-site tools for systematic monitoring of welfare of finishing pigs in order to document the level of welfare.

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1. Introduction

On the day of slaughter, stressors associated with handling, transport and lairage may have a significant impact on animal welfare and meat quality (Edwards et al., 2010a; Hambrecht et al., 2004; Staier et al., 2001). To maintain a high level of animal welfare monitoring is necessary, in order for the abattoir staff to locate and correct the cause of inexpediencies for the subsequent batch of pigs. Welfare assessment of finishing pigs at an abattoir can be carried out using the Welfare Quality® Protocol (Welfare Quality®, 2009). However, this approach is time-consuming (Dalmau et al., 2009) and is therefore not suitable for systematic monitoring of animal welfare under commercial conditions.

Recently, we used a protocol inspired by the Welfare Quality® (WQ®) and supplemented with post-mortem physiological measures to demonstrate a relationship between the handling of the pigs, their behaviour in the race to the stunning chamber and the exsanguination blood content of lactate and glucose (Brandt et al., 2013) as measures of fatigue. These results were supported by earlier findings of increased plasma lactate concentration in pigs that, compared with control pigs, experienced jamming, backing up or rearing immediately prior to slaughter (Edwards et al., 2010b). One typical measure of pig welfare at slaughter is skin damage, which has been shown to correlate with the plasma content of creatine kinase (Barton Gade, 2008; Brandt et al., 2013), the latter known to increase in humans as a result of tissue damage or muscular effort (Brancaccio et al., 2007). Hence, creatine kinase measured in the exsanguination blood may be used as an indicator of those aspects of welfare that are related to tissue and muscle damage in pigs on the day of slaughter.

Other physiological measures may also be relevant as indicators of welfare in pigs on the day of slaughter, e.g. total plasma protein...
and albumin (Averos et al., 2007; Knowles and Warriss, 2000), which are linked to dehydration and the plasma concentration of cortisol (Dokmanovic et al., 2014) which is linked to stress. Results from earlier studies suggest that physiological measures obtained relatively easily from exsanguination blood can be used to gain knowledge about animal welfare on the day of slaughter (Correa et al. 2010; Edwards et al., 2010b; Hambrecht et al., 2004). These studies demonstrated the potential of selected post-mortem measures as valid animal welfare measures. Post-mortem blood measures are non-invasive and more likely to become automated than measures obtained on live pigs due to the ease with which the samples can be collected. Physiological measures can be used as indicators of welfare when the relationship between the physiological measure and the underlying motivation is adequately validated, as emphasised by Rushen (2003). Hence, if correlations between the level of welfare on the day of slaughter measured by an animal welfare index (AWI) and physiological post-mortem measures exist, the latter may be potential candidates for systematic monitoring of animal welfare on the day of slaughter, irrespective of their biological relevance.

The aim of the present study was to investigate the relationship between selected post-mortem physiological measures and an aggregated animal welfare index (AWI). In addition the relationship between meat quality and AWI were indicated by measuring the muscle pH post-mortem. The AWI was calculated using the methodology developed by Brandt et al. (submitted), where the direct animal-based welfare measures were aggregated for each of six stages during the day of slaughter, AWIStage: pick-up pen, loading, transport, unloading, lairage and race, and across these stages, AWIOverall. We hypothesised that an AWIStage and AWIOverall would be related to the post-mortem physiological measures and, more specifically, that a relationship between skin damage score and creatine kinase activity would exist. In addition, we hypothesised that the physiological post-mortem measures could be used to detect levels of welfare above vs. below a selected threshold measured by the AWIStage/AWIOverall.

2. Materials and methods

2.1. Animals

This study involved 480 pigs from 12 different commercial herds (40 focal pigs per herd) transported to slaughter as one load from each farm. A private Danish transport company selected pig producers who had registered at least 90 pigs to the relevant abattoir on the selected experimental days. A subsample of the focal pigs (a total of n=45 pigs) from five of the commercial herds was included in the study by Brandt et al. (submitted) in order to develop the animal welfare index. Selected data from 57 other pigs have been presented by Schild et al. (2015) in a study on umbilical outpouchings. The day before slaughter, each of these 57 pigs was nose slinged for 5 min (± 1 min) for clinical examination.

In the present study, the total number of pigs delivered for slaughter from each herd was decided by the farmer and therefore differed between herds (Table 1). The pigs were transported by different commercial triple-decker Finkl lorries. The specific crosses of pig breeds used were not recorded on the farms; however, the majority of Danish slaughter pigs are crossbred Duroc (sire) and Landrace-Yorkshire (sow). The pigs were slaughtered at 100–110 kg body weight, corresponding to approximately six months of age, and the gender distribution was: 224 females, 140 castrates, 60 entire males and 56 unknown.

2.2. Study outline and design

This study was designed as an observational study (Kjaer Ersboll et al., 2004) and conducted at two standard Danish commercial abattoirs (slaughter rate: 820 pigs/h using CO2 group stunning and a slaughter line speed of 410 carcasses/h) chosen as being representative of abattoirs in Denmark.

The experiment was conducted on 12 experimental days (pigs from one herd per experimental day), selected for reasons of convenience, in November 2012 and January, April/May and June 2013. Half of the pigs were slaughtered during the winter and the other half during the spring. The two abattoirs were used equally during the two seasons. The 24-h average outdoor temperature during the experimental periods was 5 °C and 15 °C for abattoir A and −3 °C and 7 °C for abattoir B. A large private Danish transport company selected pig producers having at least 90 pigs ready for slaughter at one of the two abattoirs.

Data recording was carried out at six stages during the day of slaughter: (1) in the on-farm pick-up facilities, (2) at loading, (3) during transport, (4) at unloading, (5) during lairage and (6) in the race to the stunning chamber. In addition, skin damage was assessed the day before slaughter in the home pen, and the duration of breaks during transport was recorded (Table 1).

2.3. Procedures

Data collection was intentionally performed under commercial conditions, which is why no instructions regarding handling of the pigs were given to farmers or staff. On each farm, the 40 focal pigs were marked for individual recognition the day before slaughter and tattooed on both hindquarters for slaughter line identification. The pigs were moved from the home pens to conventional pick-up facilities by the farmer, either the day before slaughter (four herds) or in the morning of the day of slaughter (seven herds), and the pigs were therefore haphazardly mixed at this point. The duration of transport varied from 20 to 75 min (Table 1). At the abattoir, the

<table>
<thead>
<tr>
<th>Herd</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of pigs delivered per herd</td>
<td>140</td>
<td>130</td>
<td>150</td>
<td>210</td>
<td>90</td>
<td>115</td>
<td>210</td>
<td>146</td>
<td>90</td>
<td>215</td>
<td>146</td>
<td>215</td>
</tr>
<tr>
<td>No. of focal pigs</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Pick-up facility, h</td>
<td>&gt; 2</td>
<td>&lt; 2</td>
<td>&gt; 2</td>
<td>0</td>
<td>&lt; 2</td>
<td>&gt; 8</td>
<td>&lt; 2</td>
<td>&lt; 2</td>
<td>&lt; 2</td>
<td>&lt; 2</td>
<td>&lt; 2</td>
<td>&gt; 8</td>
</tr>
<tr>
<td>Loading, min</td>
<td>20</td>
<td>15</td>
<td>55</td>
<td>45</td>
<td>20</td>
<td>25</td>
<td>35</td>
<td>30</td>
<td>15</td>
<td>35</td>
<td>25</td>
<td>40</td>
</tr>
<tr>
<td>Transport, total, min</td>
<td>20</td>
<td>m</td>
<td>m</td>
<td>m</td>
<td>30</td>
<td>20</td>
<td>30</td>
<td>50</td>
<td>60</td>
<td>75</td>
<td>50</td>
<td>45</td>
</tr>
<tr>
<td>Break, total, min</td>
<td>–</td>
<td>10</td>
<td>15</td>
<td>–</td>
<td>–</td>
<td>45</td>
<td>–</td>
<td>–</td>
<td>50</td>
<td>–</td>
<td>20</td>
<td>–</td>
</tr>
<tr>
<td>Unloading, min</td>
<td>30</td>
<td>20</td>
<td>m</td>
<td>20</td>
<td>10</td>
<td>25</td>
<td>20</td>
<td>20</td>
<td>10</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Lairage, min</td>
<td>120</td>
<td>80</td>
<td>m</td>
<td>m</td>
<td>50</td>
<td>100</td>
<td>90</td>
<td>45</td>
<td>90</td>
<td>95</td>
<td>90</td>
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<tr>
<td>Race, min</td>
<td>15</td>
<td>10</td>
<td>m</td>
<td>m</td>
<td>10</td>
<td>15</td>
<td>20</td>
<td>15</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

* m=missing value.

Duration of time spent in the last three compartments prior to stunning.
90 pigs from the same farm were allocated to six identical adjoining lairage pens, each holding 15 pigs (pen area: 8.6 m², height of solid walls: 1.06 m, pigs in adjoining pens). The allocation was conducted by the haulier, abattoir personnel and a technician in accordance with abattoir procedures. It was decided to use a lairage duration of 60 min for the assessment of undisturbed behaviour and subsequently 30 min for skin damage assessment, although this depended on the need for pigs on the slaughter line (average: 92 min ± 19 min; Table 2). A lairage duration of 60 min was selected since this is typical for most Danish finishing pigs. After lairage, the pigs were moved by automatic push-hoist gates through the race at abattoir A (Automatic Driveway Systems, Btina A/S, Holbaek, Denmark). The gates moved the pigs towards and into the stunning chamber. The pigs were stunned for three minutes in groups of seven to eight in 90% CO₂ (Backloader, Butina A/S, Holbaek, Denmark), shackled and stuck in accordance with legislation (Council Regulation (EC) No. 1099/2009). Detailed descriptions of all experimental procedures can be found in Brandt et al. (submitted).

### 2.4. Data recordings

Data recordings are described in Brandt et al. (submitted) and are therefore described in summary in the present paper. Measures of the handling and behaviour of the pigs during loading, unloading and in the race to the stunning chamber were video recorded (HERO2, GoPro, ©2013 Woodman Labs, Inc., USA). At loading, one camera was fixed to the roof of the lorry, and during unloading one camera was placed at the door of the abattoir and one just above the visual veterinary inspection area. In the race to the stunning chamber, a camera was attached to the gates of each of the last three compartments. One trained observer, blinded with regard to post-mortem results, performed all of the video analyses. During lairage, the behaviour of the pigs was recorded by direct observation.

#### 2.4.1. Handling during loading and unloading

Observations of the handling and pig behaviour were performed during loading and unloading. At unloading, the recordings were initiated at the lorry ramp and terminated when the pigs had passed the visual veterinary inspection area just before entering the lairage area. The measures of handling of the animals during loading and unloading were obtained by focal animal sampling and 1–0 recording (Martin and Bateson, 2007), using a scale described by Brandt et al. (submitted) ranging from 0 to 3, in which 0 = the pig moved voluntarily or was driven by the herd, 1 = the staff used a rattle stick or board (just touching), 2 = the staff used a rattle stick, board or hand (single stroke) and 3 = the staff used a rattle stick, board or hand (repetitive strokes). For each pig, the highest score (0–3) obtained during the process was used for further analysis.

#### 2.4.2. Handling in the race

In the race to the stunning chamber, it was recorded whether the pigs were physically moved by the automatic gates (the pig was pushed forward by the automatic gate) or whether they overlapped other pigs (Correa et al., 2010) due to lack of space (Table 2). The recordings were performed continuously, with the number of events at focal pig level being counted.

#### 2.4.3. Behavioural recordings

The ethogram of pig behaviour was based on results from a previous study (Brandt et al., 2013). At loading and unloading, the behavioural recordings included the frequency of reluctance to move, turning back, slipping and falling (Table 2). Behavioural observations during lairage were conducted simultaneously by three trained observers using direct observation and instantaneous sampling (Martin and Bateson, 2007). For a total of 60 min, or until the pigs were moved to the stunning chamber, each observer, dressed in green and standing next to the lairage pens, recorded pig behaviour in two adjoining pens using observation periods of two minutes. At the initiation of each observation period, the posture of the focal pigs was recorded (standing, sitting or lying), and during the observation period the occurrence of aggression was recorded on group level (Table 2).

In the last three compartments prior to the stunning chamber, the behavioural recordings of the focal pigs included occurrence of slipping and falling (Table 2), which were recorded continuously, with the number of behaviours at focal pig level being counted.

#### 2.4.4. Clinical recordings

Skin damage was recorded four times per pig: (1) in the home pen the day before slaughter, (2) in the on-farm pick-up facility prior to loading, (3) after one hour of lairage and (4) on the slaughter line 45 min after sticking. Live skin damage assessment was performed at stages 1–3, inside the pens, on the head/ear, shoulder, middle and hindquarters, separately, using a four-point
scale (Aaslyng et al., 2013): 0: none or minor scratches, 1: few (< 3) short (up to 3 cm) scratches, 2: > 3 short or ≥ 1 long scratch (es) (> 3 cm), 3: several scratches (> 10) or skin bruising(s). On the carcasses, the assessment was performed on the shoulder, middle and hindquarters. The head/ear was not assessed at this time point due to the frequent occurrence of mechanical damage during the post-slaughter process. One experienced observer performed all of the assessments. A total skin damage score was calculated per pig as the sum of the individual scores. For live assessments, the resulting maximum possible score was 12 (3 for hindquarters, 3 for middle, 3 for shoulder and 3 for head/ear). For the post-mortem assessments, the maximum possible score was 9, since head/ear was not assessed post-mortem.

Lameness was assessed in the home pen by recording 0: no lameness and 1: uneven gait. However, due to a very low frequency (10 out of the 470 pigs), lameness was not included in the statistical analysis.

2.4.5. Physiological recordings

At exsanguination, the blood temperature of the focal pigs was measured in the sticking wound by a handheld thermologger, with the temperature logged every second (Tracksense® Pro, Ellab A/S, Hillerød, Denmark). The maximum temperature was recorded and included in the statistical analysis only when the logger had been in the sticking wound for at least 4 s.

Blood samples from the focal pigs were collected in heparin-coated tubes (VACUETTE®, 9 ml NH Sodium Heparin 16 × 100, Greiner Bio-One GmbH, Kremsmünster, Austria) and stored on ice until centrifugation (2000 rpm for 10 min) within four hours of sampling. Plasma was stored at −18 °C until analysis within 15 weeks. The plasma samples were analysed for albumin, total protein, glucose, lactate and creatine kinase (automatic biochemistry analyser, ADVIA® Chemistry Systems, Siemens, Erlangen, Germany). All intra- and inter-assay precisions were within 4% (CV).

The pH (Portamess® 913 (X) pH, Knick, Berlin, Germany) of the focal pigs was measured in the m. longissimus dorsi (LD) at the 4th–5th lumbar vertebrae 45 min (pH45) after sticking.

2.5. Animal welfare index

The welfare assessment protocol of the present study was inspired by the Welfare Quality® assessment protocol (Welfare Quality®, 2009) for finishing pigs at the slaughterhouse and has been described in detail elsewhere (Brandt et al., submitted). All AWI measures were categorised on a three-point scale (0 (mild level), 1 (moderate level), 2 (severe level)) presented in Table 3 (see Brandt et al. (submitted) for a detailed description).

In short, the weights assigned to the measures by Brandt et al. (submitted) were based on expert panel opinion, and the aggregation of the measures into an animal welfare index (AWI) was performed using a weighted linear sum of prevalence on animal level, as described by Burow et al. (2013)

\[
AWI_{\text{Stage}} = \sum_{i=1}^{6} P_i \cdot MW_i
\]

where \( P \) was the prevalence, and \( MW \) was the weight of the individual measure \( x_i \) determined by the expert panel. Six stages were included, and the number of individual measures within stages was as follows: pick-up facility: one measure; loading: seven measures; transport: two measures; unloading: six measures; lairage: five measures and race to stunning chamber: four measures.

Similarly, an animal welfare index across the six stages of the day of slaughter (AWIOverall) was calculated

\[
AWI_{\text{Overall}} = \sum_{i=1}^{6} AWI_{\text{Stage}} \cdot SW_i
\]

where \( SW \) was the weight of the individual stages determined by the expert panel.

2.6. Statistical analyses

Pig identity could be difficult to recognise, which is why missing values occurred. For ‘latency to lie down’, pigs with more than five missing values before the initial observation of lying were removed from the data set (28 pigs). For ‘latency to lie down’, ‘number of postural changes’ and ‘% time lying’, pigs with fewer than 20 observation periods (out of 30) were removed before analysis (30 pigs, 15 of which had already been removed as described above).

The AWI was calculated relative to a theoretical maximum and as such varied from 0 to 100. A value of one was added to the AWIStage per pig to avoid an AWI of zero when the AWI was log-transformed for the analysis to obtain a normal distribution of data.

Pigs with blood temperatures below 36 °C were removed as outliers (four pigs), and measures of blood temperature after less than four seconds of contact with the blood were removed (three pigs). Creatine kinase activity was log-transformed (logck) to obtain a normal distribution.

The relationship between the AWI and the physiological post-mortem measures was analysed using a linear model with logAWIStage as the response variable and the post-mortem measures (plasma concentration of lactate, glucose, logck activity, albumin, total protein) as fixed effects. Similarly, the relationship between the AWI and pH45 and blood temperature was analysed. Non-significant effects were removed by backward elimination, and least square means were reported.

The pairwise correlations between single physiological post-mortem measures and between skin damage scores and logck were calculated using Pearson’s product moment correlations.

The significance level used was \( P < 0.05 \). All statistical analyses were performed using R (R Core Team, 2014).
3. Results

3.1. Physiological post-mortem measures

The average and the range of the post-mortem measures are presented in Table 4. In general, large inter-pig variations were found.

The pairwise correlations between the physiological post-mortem measures (i.e. lactate, logck, total protein, albumin, glucose, blood temperature and pH45) were calculated in order to investigate their mutual relationships. If some measures were highly correlated, they could be pooled in the further analysis, and only one of the measures would be needed in the future. Significant positive correlations were found between lactate and logck ($r=0.15, P=0.002$), lactate and glucose ($r=0.34, P<0.0001$), albumin and logck ($r=0.13, P=0.008$) and albumin and t-protein ($r=0.53, P<0.0001$). Significant negative correlations were found between glucose and t-protein ($r=-0.41, P<0.0001$) and albumin ($r=-0.26, P<0.0001$) and between logck and t-protein ($r=-0.1, P=0.03$). In addition, logck was negatively correlated to blood temperature ($r=-0.13, P=0.04$), and pH45 was negatively correlated to lactate ($r=-0.13, P=0.048$). However, since all of the correlations were lower than 0.55, the remaining analyses were performed using the individual post-mortem measures in order to include as much information as possible.

3.2. Behavioural responses

The frequency of the measures of handling and the behavioural responses during loading, unloading and in the race are presented in Table 5. In the lairage, the average latency to lie down was 11 min (±10 min) ($n=406$), corresponding to 76% of the pigs receiving a score of 0. The average percentage of time spent lying was 63% (±24%) ($n=419$), corresponding to 72% of the pigs receiving a score of 0. The average number of postural changes was 4.4 (±2.7) ($n=419$) (from lying to either sitting or standing; changes from sitting to standing and standing to sitting were not considered), corresponding to 56% of the pigs receiving a score of 0. In total, 289 bouts of short-lasting aggression and 169 bouts of longer-lasting aggression were observed in a total of 72 pens, corresponding to an average of four bouts of short-lasting and two bouts of longer-lasting aggression per pen during the observation period of 60 min. No aggression was observed in 22 of the pens, and 23 pens received score 1 and 27 pens received score 2, corresponding to the categorisation shown in Table 3.

3.3. Clinical recordings

Significant positive correlations between the skin damage scores in the pick-up pen, after one hour in lairage, as well as post-mortem and the exsanguination plasma concentration of creatine kinase (logck) were found, although they were all below 0.50 (Table 6). The correlation coefficients became stronger the closer to slaughter the skin damage assessment took place.

3.4. AWI

The AWI can take on values from 0 to 100 and the higher the index, the more severe the welfare consequences. The average and range of the AWIs are presented in Table 7. At the individual stages, the minimum AWI was 0, although none of the pigs received an overall score of 0.

The AWIoverall was evenly distributed from 18 to 62 (Fig. 1). For loading, unloading, lairage and race, an even distribution was seen, whereas a more stepwise distribution was seen for the pick-up facility (data distributed in four groups) and the transport (data distributed in two groups) (figures not shown).

The relationship between selected physiological post-mortem measures and the AWIstage and the AWIoverall was tested by linear regression models (Table 8). There was a significant positive

Table 4

| & Average & Min & Max |
|---|---|---|---|
| Lactate, mM | 418 | 10.3 | 3.8 | 29.3 |
| CK, U/L | 418 | 7800 | 600 | 120000 |
| t-Protein, g/L | 418 | 70.7 | 57.8 | 88.6 |
| Albumin, g/L | 418 | 38.9 | 29.0 | 47.1 |
| Glucose, mM | 418 | 15.0 | 3.6 | 31.5 |
| Blood temp, °C | 262 | 38.8 | 36.0 | 40.0 |
| pH45 | 276 | 6.6 | 5.6 | 7.3 |

Table 5

The frequency of measures of handling and behavioural responses of finishing pigs on the day of slaughter presented as the percentage of pigs in which the behaviour in question was observed at least once.

<table>
<thead>
<tr>
<th></th>
<th>Loading</th>
<th>Unloading</th>
<th>Race</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence (%)</td>
<td>n=357</td>
<td>n=441</td>
<td>n=367</td>
</tr>
<tr>
<td>Slipping</td>
<td>5.9</td>
<td>7.7</td>
<td>9.0</td>
</tr>
<tr>
<td>Falling</td>
<td>3.9</td>
<td>2.7</td>
<td>17.4</td>
</tr>
<tr>
<td>Reluctance to move</td>
<td>5.3</td>
<td>1.4</td>
<td>-</td>
</tr>
<tr>
<td>Turning back</td>
<td>5.3</td>
<td>2.0</td>
<td>-</td>
</tr>
<tr>
<td>Driving</td>
<td>45.4</td>
<td>29.7</td>
<td>-</td>
</tr>
<tr>
<td>Overlapping</td>
<td>3.4</td>
<td>1.6</td>
<td>10.4</td>
</tr>
<tr>
<td>Moved by gate</td>
<td>-</td>
<td>-</td>
<td>45.0</td>
</tr>
</tbody>
</table>

- means the measure was not recorded at this stage.

Table 6

Skin damage scores of finishing pigs, obtained in the home pen, in the pick-up pen, after one hour in lairage and post-mortem using a four-point scale. The theoretical maximum for post-mortem assessment was 9 (as opposed to 12 for the live assessments).

<table>
<thead>
<tr>
<th>Facility</th>
<th>Sk in damage score, mean (min–max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Home pen</td>
<td>470</td>
</tr>
<tr>
<td>Pick-up pen</td>
<td>357</td>
</tr>
<tr>
<td>After lairage</td>
<td>413</td>
</tr>
<tr>
<td>Post-mortem</td>
<td>398</td>
</tr>
</tbody>
</table>

Table 7

The relative AWI for each stage and the relative AWIoverall for finishing pigs on the day of slaughter. The average and range of each AWI on a 0–100 scale and the number of pigs involved are presented.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Mean</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pick-up</td>
<td>317</td>
<td>9.9</td>
<td>0</td>
<td>21.1</td>
</tr>
<tr>
<td>Loading</td>
<td>357</td>
<td>8.7</td>
<td>0</td>
<td>47.1</td>
</tr>
<tr>
<td>Transport</td>
<td>360</td>
<td>1.5</td>
<td>0</td>
<td>6.8</td>
</tr>
<tr>
<td>Unloading</td>
<td>402</td>
<td>8.6</td>
<td>0</td>
<td>45.6</td>
</tr>
<tr>
<td>LAIRAGE</td>
<td>299</td>
<td>16.9</td>
<td>0</td>
<td>56.4</td>
</tr>
<tr>
<td>Race</td>
<td>367</td>
<td>7.9</td>
<td>0</td>
<td>36.4</td>
</tr>
<tr>
<td>Overall</td>
<td>103</td>
<td>39.1</td>
<td>18.4</td>
<td>62.1</td>
</tr>
</tbody>
</table>
relationship between the blood content of t-protein and AWI in the pick-up facility and during loading and a significant negative relationship between the plasma concentration of glucose and AWI during transport. For AWI during unloading, significant relationships with glucose, logck and t-protein in the exsanguination blood were found. In the race, a significant relationship between AWI and plasma concentration of lactate and content of albumin was found. For the AWIOverall, a significant relationship with plasma concentration of glucose and a tendency towards a relationship with logck were found (Table 8). All $R^2$ values were lower than 0.2, indicating that the relationships between the AWIs and the physiological post-mortem measures are weak.

Due to time constraint when conducting experiments at commercial slaughter line speed, a relatively large number of values were missing for blood temperature and pH45, which resulted in a smaller overall data set compared with the other post-mortem physiological measures. The relationship between blood temperature and AWI and between pH45 and AWI was therefore calculated in a separate model. The relationship between the AWIs and the blood temperature and between the AWIs and pH45 is presented in Table 9, which shows significant relationships between the blood temperature and AWIstage and AWIRace and between the AWILairage and pH45. It should, however, be noted that only 89 observations underlay the result for lairage.

The possibility of examining whether the physiological post-mortem measures can be used to identify AWIs categorised as ‘below threshold’ was investigated using AWIRace and the plasma concentration of lactate in the exsanguination blood as an example. A cut-off value of 25 for the AWIRace was determined from visual inspection of the data, and, based on this, indexes equal to or below 25 were categorised as ‘above threshold’, whereas indexes greater than 25 were categorised as ‘below threshold’. The

false positives and the false negatives for five different cut-off values were calculated and illustrated in Fig. 2. Based on this result, a cut-off value for the plasma concentration of lactate in the exsanguination blood of 15 was selected because the percentage of false positives and false negatives evened out.

Table 10 shows that 3.8% of the pigs had an AWIRace categorised as ‘below threshold’ but were classified as ‘above threshold’ by the plasma concentration of lactate in the exsanguination blood. These results should be considered as an initial approach to investigate the applicability of the physiological post-mortem measures in order to assess welfare on the day of slaughter. In any case, the cut-off values for a physiological measure (in this case, the plasma concentration of lactate in exsanguination blood) depend on the limits for ‘above threshold’ and ‘below threshold’ AWIs and must be balanced between levels of sensitivity and specificity. Future studies need to consider this.

Table 8
The regression coefficients and significance levels illustrated by asterisks, showing the relationship between either an animal welfare index per stage (AWIstage ) or an overall index (AWIOverall) and physiological post-mortem measures for finishing pigs on the day of slaughter. The number of animals involved in each analysis is shown. For the plasma concentration of lactate in the exsanguination blood, data were evenly distributed between 3.8 and 20.7; however, a single observation of 29.3 was also present.

<table>
<thead>
<tr>
<th>Post-mortem measures</th>
<th>$n$</th>
<th>Lactate, mM</th>
<th>Glucose, mM</th>
<th>Logck, U/l</th>
<th>t-Protein, g/l</th>
<th>Albumin, g/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pick-up</td>
<td>287</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.0078**</td>
<td>NS</td>
</tr>
<tr>
<td>Loading</td>
<td>320</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.0133*</td>
<td>NS</td>
</tr>
<tr>
<td>Transport</td>
<td>311</td>
<td>NS</td>
<td>$-0.02429^{***}$</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Unloading</td>
<td>365</td>
<td>NS</td>
<td>0.0145***</td>
<td>0.1823***</td>
<td>$-0.0094^{*}$</td>
<td>NS</td>
</tr>
<tr>
<td>Lairage</td>
<td>279</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Race</td>
<td>341</td>
<td>0.0207*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.0306**</td>
</tr>
<tr>
<td>AWIOverall</td>
<td>96</td>
<td>NS</td>
<td>$-0.3776^{*}$</td>
<td>4.8786</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

$^{***}P < 0.001; ^{**}P < 0.01; ^{*}P < 0.05; P > 0.1$. 

Table 9
The regression coefficients and significance level illustrated by asterisks, showing the relationship between the AWIs, blood temperature and pH45 for finishing pigs on the day of slaughter.

<table>
<thead>
<tr>
<th>AWIstage</th>
<th>$N$</th>
<th>Blood temperature</th>
<th>$n$</th>
<th>pH45</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pick-up</td>
<td>154</td>
<td>NS</td>
<td>173</td>
<td>NS</td>
</tr>
<tr>
<td>Loading</td>
<td>121</td>
<td>NS</td>
<td>194</td>
<td>$-0.3061$</td>
</tr>
<tr>
<td>Transport</td>
<td>101</td>
<td>NS</td>
<td>214</td>
<td>NS</td>
</tr>
<tr>
<td>Unloading</td>
<td>148</td>
<td>NS</td>
<td>235</td>
<td>NS</td>
</tr>
<tr>
<td>Lairage</td>
<td>89</td>
<td>0.2266***</td>
<td>178</td>
<td>0.3609*</td>
</tr>
<tr>
<td>Race</td>
<td>166</td>
<td>0.2201***</td>
<td>251</td>
<td>0.336</td>
</tr>
<tr>
<td>AWIOverall</td>
<td>29</td>
<td>NS</td>
<td>59</td>
<td>NS</td>
</tr>
</tbody>
</table>

$^{***}P < 0.001; ^{**}P < 0.01; ^{*}P < 0.05; P > 0.1$. 

Fig. 1. The relative AWIoverall calculated for $n = 103$ finishing pigs on the day of slaughter, illustrating the distribution on a 0–100 scale.
Significant relationships were found, for example between AWI\textsubscript{Race} and plasma concentration of lactate in the exsanguination blood, confirming earlier results which showed significant relationships between behavioural observations immediately prior to stunning and the plasma concentration of lactate at exsanguination (Brandt et al., 2013; Correa et al., 2010; Edwards et al., 2011; Edwards et al., 2010b; Hambrecht et al., 2004). Hence, the plasma concentration of lactate in the exsanguination blood, which is relatively easy to obtain in a standardised way under commercial conditions, could be a potential post-mortem indicator of the AWI in the race. Since the plasma concentration of lactate is known to increase to its maximum level within four minutes of physical exercise and to return to basal level within two hours (Anderson, 2010, cited from Correa et al., 2013), the lack of relationship between exsanguination lactate and stages prior to the race was expected. A baseline concentration of lactate of approximately 3.5 mM in whole blood (Edwards et al., 2011) and 5.1 mM in plasma (Mota-Rojas et al., 2009) have been reported, indicating that the present concentrations of lactate may have been elevated compared with baseline. In addition, CO\textsubscript{2} stunning affects the exsanguination blood concentration of lactate, since Mota-Rojas et al. (2012) found significantly increased blood concentrations of lactate in the exsanguination blood after an eight hour period of rest with access to feed and water compared to baseline and post-transportation blood concentrations. However, the prerequisite for the present study was standardised stunning procedures and thus the variation in the concentration of lactate could be attributed to handling immediately prior to slaughter, which is known to result in significantly different levels of lactate concentrations at exsanguination (Hambrecht et al. 2004). Future research should clarify which proportion of the change in lactate level that could be attributed to CO\textsubscript{2} stunning. Furthermore, Edwards et al. (2011) measured the lactate concentration at seven points on the day of slaughter and found increased concentrations, particularly after loading and at exsanguination, confirming that plasma concentration of lactate at exsanguination may be a proper indicator of welfare-related events occurring immediately prior to stunning. Thus, when blood samples are collected at only one point, measures other than the plasma concentration of lactate are needed to provide information from the other stages on the day of slaughter.

CK measured in the exsanguinated blood can be used as an indicator of events that most likely take place at any stage from farm until sticking, even with long lairage durations, since CK is reported to increase and reach its peak six hours after a stressful situation before returning to basal level 48 h later (Anderson 2010, cited from Correa et al., 2013). In the present study, a significant relationship between the plasma CK activity in the exsanguination blood and the AWI at unloading and a tendency towards a relationship between CK and the AWI\textsubscript{Overall} were found. These results are in accordance with our previous study, which reported significant correlations between exsanguination CK and handling and turning back during unloading (Brandt et al., 2013). In humans, CK increases as a result of tissue damage and muscular effort (Brancaccio et al., 2007), which supports the relationships between CK and skin damage in both the present study and previous studies (Barton Gade, 2008; Brandt et al., 2013). However, as expected, no relationship was found between CK at exsanguination and skin damage score in the home pen, since the damages are often seen after mixing with unfamiliar conspecifics (Barton Gade, 2008; Barton, 2004; Brandt et al., 2013), which did not take place until the pigs were moved to the pick-up facility. Therefore, the degree of skin damage was very low in the home pen. Throughout the day of slaughter, the correlations between CK in the exsanguination blood and skin damage score were seen, which

Table 10
This table shows the percentage-wise distribution of finishing pigs on the day of slaughter between ‘above threshold’ and ‘below threshold’ welfare based on a cut-off value of 15 for exsanguination blood lactate and 25 for AWI\textsubscript{Race}.

<table>
<thead>
<tr>
<th>Cut-off</th>
<th>AWI\textsubscript{Race}</th>
<th>≤ 25</th>
<th>&gt; 25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate</td>
<td>Above</td>
<td>Above</td>
<td>Below</td>
</tr>
<tr>
<td>≤ 15</td>
<td>Above</td>
<td>90.3%</td>
<td>3.8%</td>
</tr>
<tr>
<td>&gt; 15</td>
<td>Below</td>
<td>4.4%</td>
<td>1.5%</td>
</tr>
</tbody>
</table>

4. Discussion

The aim of this study was to investigate the relationship between an AWI\textsubscript{Stage}/AWI\textsubscript{Overall} and selected physiological post-mortem measures obtained on the day of slaughter from 480 pigs. Significant relationships between single AWI\textsubscript{Stage} and AWI\textsubscript{Overall} and the physiological measures were found, suggesting that physiological post-mortem measures may provide valuable information about the welfare of pigs on the day of slaughter.

Previous studies have used behavioural, clinical and physiological measures as indicators of animal welfare in experimental trials comparing different treatments of finishing pigs in the period from farm to slaughter (e.g. Correa et al., 2013; Correa et al., 2010; Edwards et al., 2010b; Hambrecht et al., 2004; Mota-Rojas et al., 2009). To our knowledge, the present study is among the first attempts to investigate whether an aggregated welfare assessment index can be expressed by selected post-mortem physiological measures under commercial conditions.

For both the different AWIs and the physiological post-mortem measures, large individual variations were found. The average plasma concentration of lactate in the exsanguination blood was 10.3 mM, varying from 3.8 to 29.3, in accordance with an earlier study which reported plasma concentrations ranging from 12 to 27 mM (Hambrecht et al., 2004), but is larger than the values of 6–8 mM reported by Edwards et al. (2011). These differences may be due to different methods of analysis, since Edwards et al. (2011) reported results obtained from whole blood, whereas both the present study and Hambrecht et al. (2004) reported plasma concentrations. Previously, Brandt et al. (2014) compared whole blood vs. plasma concentrations of lactate and showed that the former was generally lower than the latter, even though they were highly correlated. Similarly, Foxdal et al. (1990) showed that, in humans, the venous plasma lactate concentration was highly correlated with the venous blood lactate concentration, even though the blood lactate was approximately 40% lower than the plasma lactate.

A graphical overview of the percentage of false positive (grey) and false negative (black) responses for five different cut-off values ranging from 5 to 20, indicated by dots. A cut-off value of 25 for the AWI\textsubscript{Race} was categorised as ‘above threshold’, and indexes greater than 25 were categorised as ‘below threshold’, obtained from finishing pigs in the race to the stunning chamber. As the percentage of false positives and false negatives evened out, a lactate concentration of 15 was selected as the cut-off value.

Fig. 2.
was expected since greater muscular effort is needed during the day of slaughter and more skin damage is likely to occur when the pigs are re-mixed. Although CK may not peak until six hours later, it may increase from the occurrence of initial skin damage and onwards. Based on these findings, we suggest that the plasma concentration of CK obtained from the exsanguination blood may provide information about the welfare of pigs on the day of slaughter, particularly with regard to muscle and tissue damage. This needs to be confirmed by future larger studies involving a greater variability within the AWIs.

The plasma concentration of glucose in the exsanguination blood was included in the list of potential physiological post-mortem approximations of animal welfare from farm to slaughter, as a less specific physiological correlate to animal welfare. Accordingly, we found bidirectional relationships between the plasma concentration of glucose in the exsanguination blood and the AWIs. This was shown by a negative relationship with AWI during transport and a positive relationship with AWI during unloading as well as a negative relationship with the AWI\textsubscript{overall}. Previously, we found that the plasma concentration of glucose in the exsanguination blood was positively correlated to behaviours observed in the race to the stunning chamber (Brandt et al., 2013). Furthermore, previous studies found changes in the plasma concentration of glucose obtained at exsanguination due to transport (Averos et al., 2007; Becerril-Herrera et al., 2010) and lairage duration (Mota-Rojas et al., 2009), but, to our knowledge, no studies have so far linked behavioural and clinical observations of pigs to the plasma concentration of glucose in the exsanguination blood. Taken together, the results of the present study may suggest that the combination of the use of just one blood sample (at exsanguination) and the plasma concentration of glucose as a possible physiological indicator of animal welfare, despite the reported statistical relationships, may not be optimal, and this warrants further investigation.

As opposed to the more non-specific relationships between the plasma concentration of glucose and AWI, the plasma contents of albumin and t-proteins have been suggested as indicators of dehydration. A correlation of 0.53 between the two measures was found. In addition, a significant positive relationship between total proteins and AWI\textsubscript{pick-up} and AWI\textsubscript{loading} and a significant negative relationship with AWI\textsubscript{unloading} were found. Normal albumin concentrations in pigs range from 18 to 40 g/L (Harapin et al., 2003). In our study, the average was 38.9 g/L, indicating that some of the pigs may have experienced a certain degree of dehydration, even though the transport time was short and water was accessible during transport. Averos et al. (2007) found increased albumin when comparing sampling after unloading with sampling prior to loading and an intermediate value at exsanguination. This could be due to the fact that the pigs drank only very limited amounts of water during transport (Lambooij, 2000) and that six hours in lairage is a sufficient amount of time for the pigs to recover (Averos et al., 2007). For some reasons, the links between the expected indicators of dehydration were primarily related to the early stages of the day of slaughter (pick-up, loading, unloading) for total protein and to later stages for albumin. Hence, the use of post-mortem measures of total protein and albumin from the exsanguination blood as an indicator of animal welfare may be less promising than some of the other candidates (e.g. lactate and CK). However, future studies could focus on which measures better express the overall level of welfare and, more specifically, indicate dehydration on the day of slaughter.

Even though the duration of transport and lairage has been shown to affect blood metabolites (Becerril-Herrera et al., 2010; Dokmanovic et al., 2014; Mota-Rojas et al., 2009), in the present study the duration of transport and lairage was short and was therefore not expected to affect the blood metabolites to the same extent as previously reported. Consequently, the calculated AWIs during lairage focused primarily on the occurrence of welfare-related behaviours and, to a lesser extent, on the duration of the stages as such. However, future commercial welfare surveillance systems should take the duration of transport and lairage into account when interpreting post-mortem results obtained at exsanguination. Future research should focus on the establishment of multivariate relationships between the duration of central stages (time spent in the pick-up facility, duration of transport and lairage), the occurrence of welfare-related behaviours and the physiological post-mortem measures.

Blood temperature and pH on the slaughter line were included as measures, since previous studies found that pre-slaughter handling resulted in decreased pH 30 min after slaughter (Hambrecht et al., 2004; Van de Perre et al., 2010) and that blood temperature was related to lairage duration (Mota-Rojas et al., 2009). Furthermore, pH45 has been shown to be closely correlated to the ultimate meat quality (Steier et al., 2001), which is why possible relationships between the AWIs and post-mortem meat pH may be one way of linking animal welfare and economy. In the present study, a significant relationship between AWI\textsubscript{lairage} and blood temperature and pH45 was reported, although the measuring equipment available, combined with the slaughter line speed, resulted in a relatively high proportion of missing values for blood temperature and pH45. Hence, in order to be able to fully accept or reject pH and blood temperature as indicators of welfare on the day of slaughter, access to automatic measuring equipment capable of functioning at slaughter line speed is needed.

Other physiological measures such as cortisol may also be relevant as indicators of welfare in pigs on the day of slaughter and cortisol was, particularly some decades ago, regarded as a valid measure of animal welfare (Barnett and Hemsworth, 1990). However, cortisol was not included in the present study since the study was not designed to include baseline measures and since it is almost impossible to conclude based on one cortisol measurement because cortisol secretion follows a diurnal pattern and is pulsatile with a periodicity of approximately 90 min (Mormède et al., 2007).

In addition to the search for links between AWI and the physiological post-mortem measures, the present study suggests one method of investigating whether the post-mortem measures can be used to identify AWIs categorised as ‘below threshold’. To do this, we selected cut-off values between ‘above threshold’ and ‘below threshold’ for the AWI, based on a suggestion that a welfare index greater than 25% of the theoretical maximum was categorised as ‘below threshold’, corresponding in the present data set to approximately the 5% worse-off pigs. For the post-mortem measures (where the plasma concentration of lactate in the exsanguination blood was used as an example), the cut-off value was selected based on the distribution of false positives and false negatives as illustrated in Fig. 2. However, there could be several other approaches to setting limits for above vs. below threshold, such as the use of expert opinion and the opinion of stakeholders such as the industry or consumers to classify acceptability as done in WQ\textsuperscript{5} protocol (Veissier et al., 2011).

5. Conclusion

The present experiment calculated AWIs (both overall and for single stages) for 480 pigs, covering the last day of their life (from farm to slaughter), and investigated the relationship between an AWI\textsubscript{stage}/AWI\textsubscript{overall} and selected physiological post-mortem measures obtained from the exsanguination blood. Linear regression models resulted in significant relationships between the AWIs obtained at different stages and the post-mortem measures. However, the relationships were weak and the results need to be confirmed in
future studies. Furthermore, one method of detecting levels of welfare measured by an AWI above vs. below a certain threshold using the post-mortem measures has been suggested. However, more research is needed to determine the absolute levels of acceptable and unacceptable welfare of finishing pigs on the day of slaughter.

Taken together, these results, based on a relatively small data set and a generally high level of animal welfare, indicate that physiological post-mortem measures may provide valuable information about the welfare of pigs on the day of slaughter.

**Conflict of interest**

None of the authors have any conflict of interest to state.

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**References**

5.4 PaperIV: Welfare measurements of finishing pigs on the day of slaughter: A review. Published in Meat Science 103 (2015) 13-23

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Review

Welfare measurements of finishing pigs on the day of slaughter: A review

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Abstract

Animal welfare on the day of slaughter is of increasing concern to the authorities and consumers alike, creating a need not only to optimize the welfare of the animals but also to document the level of welfare. The day of slaughter is composed of a variety of stages, initiated when the pigs leave the home pen and including pick-up facilities, transport, lairage, stunning and sticking. At each of these stages, the animals are exposed to different stressors that, both individually and in interaction with one another, can compromise welfare. As part of the initial work aiming to document the welfare of finishing pigs on the day of slaughter, this paper provides an overview of the individual stages including a discussion of potential stressors and potential welfare measurements. These measurements are discussed with regard to their relevance and suitability for documentation of animal welfare on the day of slaughter for development of on-site tools for continuous automatic monitoring of animal welfare.

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1. Introduction

A recent EU regulation (Council Regulation (EC) No. 1099/2009) stipulates that large slaughterhouses slaughtering more than 1000 animal units per year should be able to document animal welfare. Compliance with these requirements necessitates the development of on-site tools for continuous monitoring of welfare of finishing pigs on the day of slaughter, tools that can be applied even at a high commercial slaughter line speed. Furthermore, market demands with regard to animal welfare cover the entire chain from farm to slaughter, making it relevant to also include the earlier stages (e.g. loading and transport) rather than focusing solely on the slaughterhouse.

The day of slaughter consists of a chain of potential stressors such as regrouping and housing in pick-up facilities (a separate housing unit designed to obtain maximum protection against disease), loading, transport including stops during the journey, unloading, regrouping and housing in the lairage, stunning and killing (Barton Gade, 2004). Previous studies have investigated the effect of single potential stressors within this chain, such as repeated regrouping (Coutellier et al., 2007), handling during moving (Correa et al., 2010; Edwards et al., 2010b), exposure to an unknown environment...

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(Lewis, Hulbert, & McGlone, 2008), high stocking density (Warriss, 1998), high ambient temperature (Sutherland, McDonald, & McGlone, 2009) or CO₂ concentration at stunning (Nowak, Mueffling, & Hartung, 2007; Velarde et al., 2007). However, so far no animal welfare assessment approach has considered the accumulated effects of the different potential stressors pigs encounter from the pick-up facilities at the farm until killing at the slaughterhouse.

Existing animal welfare protocols used on the day of slaughter are typically based on resource-based measurements such as the slope of the loading ramp, the number of water nipples etc. Critical control points for pre-slaughter handling have been proposed with regard to vehicle requirements, transport logistics and the health status of the animals (von Borell & Schäffer, 2005). Attention has recently been focused on the inclusion of animal-based measurements in welfare assessment protocols (Welfare Quality®, 2009). The WQ® protocol has been used to assess animal welfare on the farm (Temple, Manteca, Velarde, & Dalmau, 2011) and also from unloading at the slaughterhouse until killing, including measurements of resources, management and animals (Dalmau, Temple, Guez, Llonch, & Velarde, 2009). However, the WQ® protocol does not consider the welfare of the animals while they are at the farm pick-up units, or when they are being loaded onto vehicles on the farm or transported, which are also important potential stressors on the day of slaughter. Thus, at present there is a need to combine the whole chain of elements in an investigation that examines the suitability of biological responses for the documentation of welfare on the day of slaughter. Covering the whole chain from farm to slaughter is a challenge, since the individual incidents can interact with one another, and a certain response measured at one stage might influence the response measured at another stage.

One approach to combining protocols based on resource- and animal-based measurements was put forward by Grandin (2010), who suggested that animal welfare at slaughterhouses can be assessed using an animal-based scoring system that includes recordings of stunning efficiency, percentage rendered insensible, falls, vocalization and the use of electric prods. It was concluded that these five measurements can be used to improve animal welfare at slaughterhouses and are easy to implement and highly repeatable (Grandin, 2010). However, these recordings are labor-intensive and are therefore not recommended for continuous documentation of animal welfare at slaughterhouses. Furthermore, the five measurements can only be used at the slaughterhouse and not as documentation of the entire chain from farm to slaughter.

The development of tools for continuous automatic monitoring of animal welfare is therefore required. Furthermore, even though several measurements e.g. blood values, body temperature and meat quality traits seem highly relevant in the assessment of animal welfare on the day of slaughter (Becerril-Herrera et al., 2010; Correa et al., 2010, 2013; Hambrecht et al., 2004; Mota-Rojas et al., 2006, 2009, 2012) and have potential for automation and cost reduction, these have not yet been included in the proposed welfare schemes.

Animal welfare assessment necessitates the use of an explicit animal welfare definition, and we used the 12 criteria in the WQ® Protocol: absence of prolonged hunger, absence of prolonged thirst, comfort around resting, thermal comfort, ease of movement, absence of injuries, absence of disease, absence of pain induced by management procedures, expression of social behavior, expression of other behaviors, good human-animal relationship and positive emotional state (Welfare Quality®, 2009).

In order to facilitate the establishment of a welfare documentation system that covers as many elements of the day of slaughter as possible, our objective was to review the main elements, methods and measurements that have been used to quantify animal welfare on the day of slaughter. This review consists of eight sections covering the different stages to which pigs are exposed when destined for slaughter: the pick-up facility, loading, transport, unloading, lairage, race to the stunning chamber, stunning and sticking. The review includes potential stressors at the individual stage combined with both animal-based and resource-based potential ante- and post-mortem welfare measurements. These measurements are discussed with regard to their relevance and suitability in a documentation protocol for animal welfare on the day of slaughter followed by suggestions for possible measures with the potential for automatic recordings. The eight stages during the day of slaughter addressed in this review are summarized in the WQ® template in Table 1.

2. The pick-up facility

In Denmark, pigs are typically collected at on-farm pick-up facilities prior to arrival of the lorry. The pick-up facility is usually a separate housing unit leading directly to the loading ramp and may vary from one large outdoor pen without access to food or water to indoor pens with ad libitum access to water and the possibility of feeding. The pick-up facilities must comply with common practice such as ventilation, stocking density and floor surface. The pigs are moved from their home pen to a pick-up pen on the day before slaughter or in the morning before slaughter (Barton Gade, 2004). In this novel environment, the pigs are often mixed with unfamiliar pigs, tattooed on the hindquarters for identification and subjected to fasting. Pigs are often fasted before transport to the slaughterhouse to minimize the risk of carcass contamination by the contents of the gut (Barton Gade, 2004), and a fasting period of between five and 12 h prior to loading is recommended (Pig Research Center, 2013), which means that the pigs are seldom fed in the pick-up facilities. On farms that do not have specialized ‘pick-up’ facilities, pigs are tattooed and fasted in their home pens and are either loaded directly from these or are held in passegeways approaching the loading facilities as the load is assembled. Mixing is also common in such circumstances. Even though several potential stressors can be listed for the pick-up facilities, studies on animal welfare at this point are sparse. In the WQ® protocol, the absence of prolonged hunger (more than 12 h) and thirst is complied with by the measurements “food provision” and “water supply” (Welfare Quality®, 2009). The suggested welfare compromises at this stage defined in the WQ® criteria are summarized in Table 1.

Even though it has not been directly investigated with regard to the pick-up facilities, it is generally known that mixing of unfamiliar pigs leads to increased aggression and thereby more skin damage compared with unmixed animals (Barton Gade, 2008). Agonistic behavior and aggression are displayed when forming the hierarchy in a new group, but they are also an important part of social behavior, for example when competing for resources (Deen, 2010). The fact that the pigs are fasted in the pick-up facilities could further aggravate aggression at this stage (Brown, Knowles, Edwards, & Warriss, 1999). Aggression leads to skin damage and other physiological stress reactions (Coutellier et al., 2007), which indicates that, even though aggression is a normal behavior in pigs, it is still stressful, especially repeated aggression among the lower ranking animals. Particularly, in groups of entire males increased mounting behavior is observed (Thomsen, Bonde, Kongsted, & Rousing, 2012). Repeated introduction into novel environments and repeated regrouping may also affect resting behavior. Behavioral recordings in the pick-up facilities might therefore include mounting, aggression and posture.

A recent study on the assessment of skin damage in pigs at specific stages from the day before slaughter until slaughtering shows that one of the main stages on the day of slaughter at which skin damage occurs in finishing pigs is in the pick-up facilities (Aaslyng, Brandt, Blaabjerg, & Staier, 2013). Skin damage can be assessed on both the live pig and the carcass, and three-, four- and five-point scales have been developed and documented (Aaslyng et al., 2013). It has been recommended to assess skin damage at the slaughter line 45 min post mortem, since damage is more easily seen on the carcass than on the live pig (Barton Gade, Warriss, Brown, & Lambooj, 1996). However, the assessment of skin
Table 1
The eight stages on the day of slaughter and their relevance to animal welfare defined by the Welfare Quality® protocol.

<table>
<thead>
<tr>
<th>WQ® criteria</th>
<th>Pick-up</th>
<th>Loading</th>
<th>Transport</th>
<th>Unloading</th>
<th>Lairage</th>
<th>Race to stunning</th>
<th>Stunning</th>
<th>Sticking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hunger</td>
<td>Duration of food deprivation</td>
<td>–</td>
<td>Duration of food deprivation</td>
<td>–</td>
<td>Duration of food deprivation</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Thirst</td>
<td>Duration of water deprivation, signs of dehydration</td>
<td>–</td>
<td>Duration of water deprivation, signs of dehydration</td>
<td>–</td>
<td>Duration of water deprivation, signs of dehydration</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Resting comfort</td>
<td>Latency to lie down, posture changes</td>
<td>–</td>
<td>Latency to lie down, posture changes</td>
<td>–</td>
<td>Latency to lie down, posture changes</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Thermal comfort</td>
<td>Air temperature, humidity, draft</td>
<td>–</td>
<td>Air temperature, humidity, draft</td>
<td>–</td>
<td>Air temperature, humidity, draft</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ease of movement</td>
<td>Space allowance, group size</td>
<td>–</td>
<td>Space allowance, group size</td>
<td>–</td>
<td>Space allowance, group size</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Injuries</td>
<td>Mixing, re-grouping, fighting wounds</td>
<td>–</td>
<td>Mixing, re-grouping, fighting wounds</td>
<td>–</td>
<td>Mixing, re-grouping, fighting wounds</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Disease</td>
<td>Fitness for transport</td>
<td>–</td>
<td>Fitness after transport</td>
<td>–</td>
<td>Fitness after transport</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Pain induced by humans</td>
<td>Quantity and quality of tattooing, quantity and quality of driving</td>
<td>–</td>
<td>Quantity and quality of driving, vibrations, positive and negative accelerations</td>
<td>–</td>
<td>Quantity and quality of driving, vibrations, positive and negative accelerations</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Social behavior</td>
<td>Mixing, re-grouping</td>
<td>–</td>
<td>Mixing, re-grouping</td>
<td>–</td>
<td>Mixing, re-grouping</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Other behavior</td>
<td>Aggressive behavior</td>
<td>–</td>
<td>Aggressive behavior</td>
<td>–</td>
<td>Aggressive behavior</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Positive emotions</td>
<td></td>
<td>–</td>
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<td>–</td>
</tr>
</tbody>
</table>

damage on the carcass does not specifically reflect the pick-up facilities, but rather describes the sum of occurrences throughout the day of slaughter.

Physiological measurements of the adrenocortical activity (cortisol) or dehydration (albumin) can be used, although the adrenocortical hormones in particular need to be sampled directly after the pick-up area and cannot be sampled post mortem. This makes e.g. cortisol a less obvious candidate for automated documentation of welfare at this specific point. Dehydration can be assessed by measuring the osmolality of the blood (Broom, 2000; Knowles & Warriss, 2000) or by using the blood concentration of albumin as an indicator (Knowles & Warriss, 2000). These measurements are not specific for the pick-up facilities, but they describe thirst throughout the entire day of slaughter.

Haptoglobin (HAP) is an acute phase protein, and the blood level increases when an animal is exposed to infection and/or stress in general. HAP has been measured in the exsanguinated blood and was evaluated as an indicator of management and housing scores, not specifically in the pick-up facilities but on the farm in general (Geers et al., 2003). It was suggested that HAP could be used as an indicator of animal welfare, with good on-farm scores resulting in significantly lower HAP at slaughter (Geers et al., 2003).

In summary, the welfare of finishing pigs in the pick-up facility can be compromised, especially due to the novelty of the environment, mixing of novel conspecifics and the possibility of thirst and hunger. At this specific site, the welfare can be quantified by animal-based measurements such as aggression or the clinical examination of skin damage. In addition, the housing facilities, such as access to water and food, ventilation, stocking density and floor surface can be recorded.

3. Loading

During loading, the pigs are again moved to a novel environment and are mixed or remixed, depending on the pick-up facilities, with unfamiliar conspecifics (Barton Gade, 2004). Driving by the handlers may stress the pigs, the extent to which depends very much on the attitude of the farmer and the haulier (Barton Gade, 2004). This can be quantified by behavioral observations of the pigs, for example turns, stops, etc., and of the handler, for example use of an electric prod, use of paddle on the walls or on the pigs, etc. The possible compromises of animal welfare at this stage defined in the WQ® criteria are summarized in Table 1.

Behavioral recordings using video surveillance from leaving the pen until loading onto the lorry for comparison of three driving devices consisted of frequencies of slips/falls, overlap, 180° turns, attempts at turning, reluctance to move forward, quantity and quality of handler interventions, and vocalizations (Correa et al., 2010). Compared with using a board and a paddle and/or a compressed air prod, the use of an electric prod resulted in more falls, fewer stops and less turning around, more and longer vocalizations, greater heart rate, greater blood lactate concentrations at exsanguination, greater ultimate pH values and greater incidence of blood-splashed hams (Correa et al., 2010). In this study, more pigs turned around when not prodded, and therefore handling the pigs in a more friendly manner may result in more frequent turning around. Reluctance to move forward and turn around should therefore be interpreted context-specifically and with caution.

Elevated heart rate can be an indicator of changes in the physiological state of an animal; however, physical activity influences heart rate and should be taken into account when evaluating heart rate measurements. Heart rate was significantly affected by the moving device used during loading in the study by Correa et al. (2010). Furthermore, introduction into a new environment resulted in an elevated heart rate (Lewis et al., 2008), and moving groups of more than five pigs also resulted in an elevated heart rate (Lewis & McGlone, 2007). Measuring heart rate variability (HRV) and analyzing the variability in the intervals between heartbeats may be useful in evaluating animal welfare (von Borell et al., 2007). Measuring the heart rate is useful for research purposes since it measures the physiological state of the animal continuously in a non-invasive way. It is, however, unsuitable for continuous monitoring of animal welfare for documentation purposes because of lack of feasibility.

In addition to the handling of the pigs, other potential stressors are present during loading: noise, darkness/light, slippery floors and the slope of the ramp are unfamiliar conditions for many pigs (Grandin, 2000; von Borell & Schäffer, 2005). Furthermore, the weather
may influence the loading of the pigs e.g. rain, wind or strong sunlight may cause the pigs to stop when entering the door leaving the building. This can be measured either by behavioral observations of the pigs, such as the frequency of slipping/falling and reluctance to move forward, or by measurements of the surroundings, such as the degree of the slope of the ramp, and correlating these with knowledge of the effect on the animal.

In summary, loading on the farm can potentially compromise the welfare of the pigs due to both handling and external factors. It is therefore highly relevant to include this in an assessment of the welfare on the day of slaughter, even though this is not the case in most welfare schemes today. The welfare of finishing pigs during loading can be assessed by animal-based measurements such as observations of slipping and falling and reluctance to move on to the lorry, possibly combined with measurements of heart rate and vocalization. Furthermore, the transport and handling facilities including the floor surface, the slope of the ramp and whether or not there is protection against weather conditions can be recorded.

4. Transport

Transport to the slaughterhouse can be divided into short journeys, defined as a maximum of 8 h, and long journeys, defined in the EU as 8 h or more. In Denmark, the Netherlands and Belgium, most pigs are transported in triple-decker lorries, although the farmers are also seen taking the pigs to the slaughterhouse themselves in smaller vehicles. In the rest of Europe, double-decker lorries are common, while in North America double-decker pot-belly lorries are often seen. In Asia, small lorries are most commonly used. The type of lorry used might influence the effect of transport time and other factors of relevance to the welfare of the animals during transport. In order to comply with EU legislation (Council Regulation (EC) No. 1/2005), animals must be suited for the intended transport. Pigs that are unable to walk may not be transported, and pigs that are unable to walk on arrival at the slaughterhouse and pigs with severe open wounds or prolapses must be killed on arrival. The possible compromises of animal welfare at this stage defined in the WQ® criteria are summarized in Table 1.

The effect of transport on animal welfare must be seen as a multifactorial challenge, for which a combination of stressors rather than a single factor is responsible for animal welfare, as described by Schwartzkopf-Genswein et al. (2012). The factors during transport that may compromise pig welfare are journey duration and ambient temperature (Sutherland et al., 2009), placement on the transporter (Warriss, 1998; Warriss et al., 2006), stocking density (Vink & ter Beek, 2008; Warriss et al., 1998), vibrations (Warriss, 1998), floor type and bedding (Sutherland et al., 2009) and mixing with unfamiliar pigs. Furthermore, Nielsen, Dybkjær, and Herskin (2011) reviewed farm animal welfare in relation to transport duration and stated that it is not the journey duration itself that causes concern in relation to animal welfare but rather the associated negative aspects such as feed and water deprivation, temperature and lack of opportunity to rest. However, these negative aspects are by their nature related to the length of the journey as recently reviewed by Miranda-de la Lama, Villarroel, and Maria (2014).

Mortality during transport is the ultimate welfare burden. Barton Gade, Christensen, Baltzer, and Petersen (2007) found that mortality increased from 0.0016% to 0.0223% when comparing transport distances in Denmark of 50 km with distances of >200 km. This is supported by results from Gosalvez, Averos, Valdelvira, and Herranz (2006), who compared distances of 50 km with distances of >100 km in Spain and found an increase in mortality from 0.21% to 0.46%. It has, however, been argued that the quality of the transport, including the selection of animals fit for transportation, road conditions, vehicle design and operation, space allowance, temperature and ventilation is of greater importance than the duration (Cockram, 2007). Likewise, it has been shown that the risk of mortality during transport is doubled when the pigs are not fasted prior to loading (Averos, Knowles, Brown, Warriss, & Gosalvez, 2008). Another survey study of pigs slaughtered at one slaughterhouse in the US found that mortality increased with journey time for journeys lasting less than 4 h but tended to decrease with longer journeys (Sutherland et al., 2009). Sutherland et al. (2009) suggested that pigs that died on shorter journeys were unable to recover from loading stress, although the pigs’ lack of suitability for transportation, rather than the transport duration, could also explain the higher mortality rate on arrival. Furthermore, the number of farms from which the pigs were collected negatively affected mortality. Collecting pigs from more than one farm significantly increased mortality compared with collecting pigs from only one farm, suggesting that other factors such as mixing of pigs from different farms may have greater significance for pig welfare than the distance (Gosalvez et al., 2006).

Transport duration affects the welfare of pigs, meat quality traits and mortality. Transport durations of 8 h resulted in an increased number of pigs with a pH below 5.7 45 min after slaughter compared with transport durations of 16 and 24 h and an increased number of pigs with a pH above 6.3 when transported for 24 h compared with 8 h in Pietrain LY crosses (Mota-Rojas et al., 2006). Furthermore, journeys lasting eight and 16 h resulted in significantly increased blood glucose, lactate and hamatocrit levels compared with baseline measurements, and 8-hour journeys resulted in a higher glucose concentration compared with 16-hour journeys, whereas lactate concentrations were higher for 16-hour journeys in Pietrain LY crosses (Becerril-Herrera et al., 2010). This indicates exhaustion in pigs subjected to increasing transport times from at least 8 h. Since 8-hour journeys were not compared with shorter journeys, exhaustion might occur earlier.

Motion sickness is a condition relevant to the transport on the day of slaughter and is relevant to the welfare criterion “absence of disease”. The plasma concentration of vasopressin is a useful indicator of motion sickness (Broom, 2000; Knowles & Warriss, 2000). A measurement of the G-force taken during the transport simultaneously with video surveillance showed that, when traveling at 70 km/h and fully applying the brakes, triggering the ABS anti-blocking system, the G-force in the forward direction was 0.6 g. With a loading density of 100 kg per 0.42 m², this braking forced the pigs forward in the pen, and those not leaning against anything lost their balance. This happened again when the brakes were re-engaged or when the truck came to a complete stop (Nøddegaard & Brusaard, 2004).

Stocking density during transport or during lairage may have an impact on the welfare of pigs. Four stocking densities (0.35, 0.39, 0.42 and 0.50 m² per 100 kg pig) during journeys lasting 2–3 h were compared. Pigs transported at 0.50 m² had a significantly lower creatine kinase activity U/L (CK) in blood samples compared with the other stocking densities, although no difference in the plasma concentration of lactate obtained at exsanguination was found (Barton Gade & Christensen, 1998). Pigs transported at 0.35 m² spent more time lying down during the journey than pigs transported at 0.42 and 0.50 m² (Barton Gade & Christensen, 1998). Furthermore, high stocking density (<0.42 m²/100 kg) (Warriss, 1998) increases mortality during transport. Another study with 2496 pigs compared a stocking density of between 0.31 and 0.5 m²/100 kg for a journey lasting between 150 and 260 min followed by 1 h of lairage. No effect was seen of stocking density on either meat quality (pH and reflectance) or skin damage (Guise et al., 1998). The EU regulation requires 0.42 m² per 100 kg pig. An assessment of the number of pigs on each lorry could be used as documentation of compliance with this requirement, even though it does not express the level of welfare of the individual animal.

“Comfort around resting” is one of the WQ® criteria for good welfare. This is assessed by scoring the bedding in the truck. According to EU legislation, bedding is mandatory in the truck (Council Regulation (EC) No. 1/2005). Bedding may improve comfort around resting as long
as the bedding is dry. In addition, dry bedding lowers the thermoneutral zone of the pigs and may affect the need for ventilation. However, to the best of our knowledge, no scientific comparison of bedding materials is described in the literature, and a systematic comparison of different types and amounts of beddings is desirable.

Pigs are sensitive to high ambient temperatures (Knowles & Warriss, 2000), and, since pigs cannot sweat, they must rely on other means of thermoregulation, such as moving away from the heat source, changing posture and/or wallowing (Knowles & Warriss, 2000). However, when confined to a certain area such as a lorry, particularly during the hot season in which temperatures in the pot-belly exceeding 30 °C have been reported, the thermal comfort of the pigs is dependent on adequate air renewal or water sprinkling systems producing a mist (Schwartzkopf-Genswein et al., 2012).

The installation of forced ventilation combined with a misting system may improve animal welfare in terms of reduced risk of heat stress during the hot seasons (Barton Gade et al., 2007). Furthermore, installation of ventilation and mist systems that automatically start when the temperature rises above 20 °C for the ventilation and 25 °C for the mist system have been recommended (Christensen, Blaabjerg, & Hartung, 2007). Water sprinkling during transport at an ambient temperature below 23 °C in a pot-belly trailer resulted in more pigs standing during transport and more pigs lying down during lairage compared to no sprinkling during transport. Non-sprinkled pigs spent more time lying down during transport and more time drinking during lairage (Fox et al., 2014). Furthermore, sprinkled pigs had significantly lower exsanguination blood lactate levels independent of the ambient temperature which could be due to less physical activity and pH 1 h post mortem was greater, however, no difference in pH 24 h was found (Nannoni et al., 2014). Temperatures above 20 °C recorded in the lorry on arrival at the slaughter plant significantly affected the percentage of dead pigs on arrival compared with lower temperatures (Sutherland et al., 2009). Thus, water sprinkling can improve pig welfare particularly at high ambient temperatures.

Stops during the journey increased the mortality rate, most probably due to an increase in temperature in the lorry (Christensen et al., 2007). When the vehicle is moving, natural ventilation is sufficient in summer conditions in the UK (Warriss et al., 2006); however, when the vehicle comes to a stop, the temperature in the lorry rises, and this may have a detrimental effect on the welfare of the animal.

It has been suggested that prolonged increases in body temperature could be used as an indicator of welfare (Broom, 2000) and particularly as an indicator of stress during transport (Knowles & Warriss, 2000), but it is difficult to measure continuously under commercial conditions. Instead, the temperature of the pigs on arrival could be an indicator, although, since temperature needs to be measured behind the ears or in the eyes (Schmidt et al., 2013), this could be a challenge. In the WQ® protocol, thermoregulatory behaviors of the pigs, such as shivering, panting and huddling, are included; however, these measurements are not used in the reviewed literature and are difficult to record during transport.

Indicators for the overall burden on the animals during transport could be found through an analysis of blood samples taken at exsanguination. Blood samples were taken from 48 pigs transported for 2 h, before and after transport (Mota-Rojas et al., 2009). The journey resulted in significantly increased blood glucose, lactate and albumin values as well as increased creatine kinase activity between the samples (Table 2), even though the values were not dependent on the various events during transport, but rather on the transport itself (Mota-Rojas et al., 2009).

In another study, blood samples were taken before loading, after unloading and at exsanguination from pigs transported for either 1 h or 13 h under commercial conditions in Spain during either the summer or winter and housed in lairage for 6 h (Averos, Herranz, Sánchez, Comella, & Cosálvez, 2007). The study showed an increase in blood values of CPK, LDH, total protein, albumin and cortisol from loading until unloading and a decrease from unloading until exsanguination (Table 3) (Averos et al., 2007). There was no difference in glucose concentration between loading and unloading, although there was an increase at exsanguination (Averos et al., 2007). This indicates that the resting period at the slaughterhouse allowed the majority of the blood parameters to decrease, although some of the measurements were affected by journey duration and season. CPK levels were significantly affected by season, with higher activities during the winter, and the concentration of glucose was higher on short journeys (Averos et al., 2007). This indicates that, if blood values of selected metabolites are used as documentation of animal welfare, the values should be corrected for lairage time.

Absence of prolonged hunger and thirst is included in the WQ® criteria. When pigs are transported in Denmark, water must be provided, even though previous studies have demonstrated that pigs consume limited amounts of water during transport (Lambooj, 2000). As mentioned previously, albumin concentration in the blood can be an indicator of thirst, just like osmolality and total protein (Averos et al., 2007; Knowles & Warriss, 2000). In Table 4 some normal values of selected serum variables are listed.

The normal range of serum albumin in pigs is 23–40 g/L (The Merck Veterinary Manual, 2012), and values for domestic pigs of between 18 and 40 g/L were reported by (Harapin, Bedrica, Hahn, Sostarie, & Gracner, 2003). For humans, normal levels range between 34 and 54 g/L, and increased values may be due to dehydration (MedlinePlus, 2013). This indicates that the pigs in Table 3 were slightly dehydrated on the day of slaughter.

A system for on-line surveillance of animal transports has previously been developed (Geers et al., 1998). The system included the recording of animal identification, body temperature and geographical position and was developed to monitor animal diseases, though it may also be useful for monitoring welfare during transport (Geers et al., 1998). However, the system may not be feasible for welfare monitoring of slaughter pigs at this point because the system requires electronic ear tags and implanted transmitters for temperature measurements. Similarly, an implantable data logging system has been developed in order to monitor deep body temperature of pigs during commercial transport (Mitchell, Kettlewell, Villarreal, Farish, & Harper, 2010). If electronic ear tags are introduced to commercial pig production enterprises, continuous monitoring using electronic identification may become realistic. Furthermore, cheap video technology and computer

<table>
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<tr>
<th>Table 2</th>
<th>Plasma profile before and after 2-hour transportation (Mota-Rojas et al., 2009).</th>
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<tbody>
<tr>
<td>Compound</td>
<td>Before transport</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>90.25 ± 1.09</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>5.10 ± 0.21</td>
</tr>
<tr>
<td>CK (U/L)</td>
<td>465.62 ± 5.82</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>24.08 ± 0.45</td>
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<tr>
<th>Table 3</th>
<th>Overall effect of journey (1–13 h) and lairage for 6 h on serum variables of pigs transported to slaughter (Averos et al., 2007).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loading</td>
<td>Unloading</td>
</tr>
<tr>
<td>Cortisol (μg/dL)</td>
<td>3.47 ± 0.19</td>
</tr>
<tr>
<td>Glucose (g/L)</td>
<td>0.54 ± 0.03</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>1145 ± 71.58</td>
</tr>
<tr>
<td>CPK (U/L)</td>
<td>2337 ± 377</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>41.83 ± 0.51</td>
</tr>
<tr>
<td>Total proteins (g/L)</td>
<td>72.23 ± 0.80</td>
</tr>
</tbody>
</table>

*Means ± S.E. with different subscript in rows are different (p < 0.001).
image processing that can perform automatic recognition of animal behavior may be useful in the future assessment of pig welfare. In summary, animal welfare during transport can be compromised by external factors such as journey duration, temperature and stocking density. Many of these aspects have been investigated widely, whereas topics such as the transport of unsuitable pigs and bedding in the trucks require further investigation. The welfare of finishing pigs during transport can be assessed by animal-based measurements, such as blood sampling at exsanguination, to determine the level of, for example, vasopressin (motion sickness), albumin (thirst and hunger) or lactate and glucose (energy metabolism). Lairage duration and time of last feeding should also be taken into account when evaluating these measurements. Resource-based measurements, such as temperature in the truck and automatic control of ventilation above or below a certain temperature, could also be used.

5. Unloading

After arriving at the slaughterhouse, the pigs are unloaded from the truck and moved to a lairage pen. Again, the pigs are moved through a new environment, giving rise to several potentially stressful situations both due to the physical layout of the slaughterhouse, such as the angle of the unloading ramp, and due to human-animal interactions, including the use of driving devices (Van de Perre, Permentier, De Bie, Verbeke, & Geers, 2010). However, this stage of the day of slaughter has only been sparsely investigated. The unloading and moving of the pigs to the lairage pens are typically performed by the slaughterhouse staff, who, in the EU, must have specific training (Council Regulation (EC) No. 1099/2009). Furthermore, all animals must be inspected by the veterinary authorities before the pigs are put into lairage pens. The possible compromises on animal welfare at this stage defined in the WQ® criteria are summarized in Table 1.

Another important consideration associated with unloading is the amount of time the pigs have to wait in the truck at the slaughterhouse prior to being unloaded. Increasing the waiting time from zero to more than 4 h at the slaughter plant prior to unloading in trailers could also be determined by resource-based measurements such as temperature in the truck and automatic control of ventilation above or below a certain temperature, could also be used.

6. Lairage

The main purpose of the lairage is to ensure a continuous supply of pigs to the slaughter line. Previously, the lairage time also served as a recovery time for the pigs after the transport, but it has been shown that this is less important in pig populations without the halothane gene (Aaslyng & Barton Gade, 2001). In the lairage pens, the pigs are sometimes mixed with new conspecifics, which is a potential stressor. The number of water nipples, the pen size, stocking density and floor type are also important for the welfare at this stage of the day of slaughter. The possible compromises on animal welfare at this stage defined in the WQ® criteria are summarized in Table 1.

In the WQ® protocol, “comfort around resting” is assessed in the lairage by scoring the floor condition. According to EU legislation, bedding and feeding is mandatory when pigs are housed at the slaughterhouse for more than 12 h (Council Regulation (EC) No. 1099/2009). Nevertheless, ‘comfort around resting’ could also be determined by measuring lying behavior at lairage. Rabaste et al. (2007) found that pigs in smaller groups of 10 compared with 30 pigs tended to spend more time lying down. However, it is difficult to interpret the underlying motivation of lying behavior in lairage pens simply from behavioral studies, since there may be several causes. Pigs may lie down either in order to relax or as a result of fatigue.

WQ® does not include any measurements that refer to the criteria ‘expression of social behaviors’ and ‘expression of other behaviors’ on the day of slaughter. Potentially relevant measurements include lying behavior, mixing of unfamiliar pigs and aggressive behavior. Mixing of unfamiliar conspecifics may increase the incidence of aggression, particularly when entire males are included (Barton Gade, 2004). Mixing of entire males in the lairage is also expected to increase sexual behavior. Skin damage increases during lairage (Aaslyng et al., 2013) because of mixing but also due to inappropriate management procedures such as the use of electric goads and sticks (Faucitano, 2001) when moving the pigs out of the lairage pens towards the stunning chamber. Rabaste et al. (2007) found that keeping pigs in groups of 30 resulted in ten times more aggression...
than when kept in groups of ten. An increased level of aggression in larger groups could also lead to an increased level of skin damage. In addition, mixing of unfamiliar pigs in the lairage increases CK levels compared with keeping pigs in stable groups on the day of slaughter, and plasma CK was positively correlated with skin damage scores on the ham and shoulder (Barton Gade, 2008).

The importance of the lairage time has been studied extensively. One study investigated skin damage in Dutch slaughterhouses and found that the level of skin damage increased with increasing lairage duration from 0 to 3 h and with increasing stocking density from 1.0 to 2.7 pig/m² (Geverink, Engel, Lambooj, & Wiegant, 1996). In accordance, skin damage score recorded in an observational study increased with lairage time from one to 15 h (Guárdia et al., 2009). Geverink et al. (1996) found a significantly longer duration of agonistic behavior after 40–70 min lairage time compared with the first ten minutes of lairage and no difference between 80 and 90 min and the first 10 min. Clearly, a longer lairage duration allows for rest but may increase the risk of aggression and thereby skin damage as reviewed by Faucitano (2010).

Not only the skin damage but also the metabolic rate is affected by lairage. In a study comparing 0 and 5 h lairage combined with electrical stunning, blood samples were taken before and after lairage and after exsanguination (Mota-Rojas et al., 2009). Significant differences were found in glucose, lactate and albumin concentrations, which were higher in non-rested pigs, whereas CK was higher in rested pigs after lairage. At exsanguination, glucose, lactate, albumin and CK were all higher in non-rested pigs. Furthermore, non-rested pigs had a higher blood temperature at exsanguination, while pH 45 min was higher in rested pigs and temp 45 min was lower (Mota-Rojas et al., 2009). Increased albumin could indicate dehydration and increased glucose and lactate could indicate increased metabolic rate and fatigue. The explanation for higher CK in rested pigs could be that increased lairage time may increase the incidence of skin damage which release CK.

Blood samples collected before transport and at exsanguination for pigs slaughtered on arrival were compared between pigs slaughtered directly and pigs given a 24-hour rest period with free access to water (Smiecinska, Denaburski, & Sobotka, 2011). The journey caused a stress response measured by elevated CK activity and cortisol levels in pigs slaughtered directly after transport. Pigs given a 24-hour rest period had lower cortisol levels at slaughter, whereas the rest period did not affect CK levels at slaughter (Smiecinska et al., 2011). Cortisol decreases within 1 h (Choi, Jung, Choe, & Kim, 2012), whereas CK levels remain elevated for 24 h after exercise in humans (Brancaccio, Maffulli, & Limongelli, 2007). These studies indicate that rested pigs recover from the journey during the lairage period. However, this does not mean that the welfare of these pigs is better, since all pigs suffer from the same degree of exhaustion — the rested pigs recover before slaughter, while the other group of pigs do not recover; however, the maximum burden on the pigs is not necessarily higher in the non-rested group.

In summary, the welfare of finishing pigs during lairage can be compromised by mixing and excessively large groups. It can be measured by assessment of resting behavior, aggression, skin damage and exsanguinated blood glucose, lactate, albumin and CK, blood temperature, as well as post mortem loin temperature and pH.

7. Race to the stunning chamber

After lairage, the pigs are moved to the stunning chamber. This can be done manually, implying a human-animal relationship, or by automatic push-hoist gates. The push-hoist gate system can be used to ensure compliance with slaughter line speed, although it is important that the gates are finely adjusted, so that they stop, and do not drag an immobile pig (Barton Gade, 2004). In some systems, the pigs are moved into a single-file chute immediately prior to stunning. Since pigs are herd animals, this is against their nature and, depending on the system, they need to be forced forward, for example by using electric prods. A limited use of electric prods is currently allowed on adult animals just before stunning (Council Regulation (EC) No. 1099/2009). Other systems use group stunning CO₂ systems, in which the pigs are moved into the stunning chamber in groups of five to eight pigs, using their natural group behavior (Barton Gade, Blaabjerg, & Christensen, 1992, 1995; Christensen & Barton Gade, 1997; Steier, Aaslyng, Olsen, & Henckel, 2001). Table 1 summarizes the possible burden on the pigs with regard to the WQ® criteria for animal welfare.

Pigs that are forced forward are known to vocalize. Exsanguinated blood lactate and CPK (creatine phosphokinase) are related to vocalizations prior to stunning (Warriss, Brown, Adams, & Corlett, 1994). Comparison between group stunning and traditional single-chute stunning showed a higher concentration of creatine phosphate in meat samples taken directly from the pigs after the group stunning system, indicating a lower metabolic rate resulting from a lower stress level in the group system (Steier et al., 2001). In another study, pigs were observed moving through a crowd pen and a single-file chute, and the concentration of exsanguinated blood lactate was compared between pigs experiencing falls, jamming, rearing, turning back, backing up and vocalization and pigs experiencing none of those events (Edwards et al., 2010a). Vocalization, jamming and backing up resulted in a significant increase in the concentration of lactate, and it was concluded that blood lactate concentration could be used to monitor animal welfare (Edwards et al., 2010a). Similar results were found in a later study (Brandt et al., 2013), which found a correlation between events taking place immediately prior to stunning and blood levels of glucose.

Similarly, pigs stressed by being forced with electric prods to move back and forth four times prior to stunning were compared with pigs that were handled without the use of electric prods and that walked directly to the stunning area (Hambrecht et al., 2004). The use of electric prods resulted in significantly increased cortisol and lactate exsanguination concentrations and a more rapid decrease in pH measured 30 min after slaughter. There are, however, many factors other than pre-slaughter stress that can affect ultimate pH, such as scalding and chilling, and it may therefore be advantageous to measure the parameters for welfare assessment before the scalding process (Hambrecht et al., 2004). It was therefore concluded that blood lactate could be a good indicator of physical and psychological stress associated with pre-slaughter handling (Hambrecht et al., 2004).

Thermal images from the inside of the pigs’ ears were compared with temperature, cortisol concentration and creatine kinase (CK) activity in the blood at exsanguination. There was a significant correlation between cortisol concentration and blood temperature and between CK and ear temperature. Furthermore, there was a similar, though non-significant, relationship between cortisol and ear temperature (Warriss, Pope, et al., 2006). It was suggested that thermal imaging cameras could be used to monitor the physiological state of the pigs continuously and thereby be used as an indicator of their welfare (Warriss, Pope, et al., 2006).

In summary, the welfare of finishing pigs in the race to the stunning chamber can be assessed by behavioral measurements such as falls, jamming, rearing, turning back, backing up as well as vocalizations. Furthermore, exsanguination blood glucose, lactate, blood and ear temperature, as well as post mortem loin temperature and ultimate pH can be used as indicators of welfare in the race to the stunning chamber knowing that the processing may influence these results.

8. Stunning

Pigs must be stunned before sticking according to the EU regulation (Council Regulation (EC) No. 1099/2009). The purpose of this is to induce insensibility to pain until the animal is dead (McKinstry & Anil, 2004), and this is therefore relevant to the welfare criterion “absence of pain due to management procedures”. However, suboptimal stunning can induce pain, and the handling prior to stunning can also involve fear and pain. Therefore, optimal handling prior to stunning combined with the correct stunning procedure is crucial to the welfare...
of the animals at this stage of the day of slaughter. Possible compromises on animal welfare at this stage defined in the WQ® criteria are summarized in Table 1.

For pigs, the most common methods used are electrical stunning and gas stunning. Electrical stunning requires the animal to be restrained, which is a potential stressor. Stress at restraining immediately prior to stunning can be assessed by measuring vocalizations (Warriss et al., 1994). Electrical stunning induces immediate unconsciousness (Troeger, 2008), which can be an advantage in terms of animal welfare, although it is crucial that the electrodes are positioned correctly in order to achieve the intended stunning (Velarde, Gispert, Faustiano, Diestre, & Manteca, 2000). In a survey in the UK, 15.6% of all the pigs were incorrectly stunned the first time, and the stunning procedure had to be repeated (McKinstry & Anil, 2004). This must be regarded as being highly detrimental to the welfare of the animals. Another drawback of electrical stunning is an increased number of blood splashes in the meat and a reduced pH, although these are not relevant to animal welfare (Velarde, Gispert, Faustiano, Manteca & Diestre, 2000; Velarde et al., 2001).

CO2 is used as a stunning gas in many parts of the world. It has, however, been the subject of some discussion since the pigs show signs of aversion to the gas a few seconds before unconsciousness occurs (Dalmau, Rodriguez, Llonch, & Velarde, 2010; Llonch, Dalmau, Rodriguez, Manteca, & Velarde, 2012). This is relevant to the welfare quality criterion “absence of fear”. Argon has been suggested as an alternative, but since the pigs remain unconscious for less time, it would risk compromising welfare, since the pigs are not in a state of deep unconsciousness at stunning. Different concentrations of CO2 have also been compared, and stunning in a deep lift system with 80% CO2 for at least 100 s can be regarded as acceptable in terms of animal welfare, assessed by clinical signs of consciousness (Nowak et al., 2007). The EU demands at least 40% CO2 (single concentration) or at least 40% followed by a higher concentration (two-phase CO2) (Council Regulation (EC) No. 1099/2009). A comparison of 70% CO2 stunning for 60 s with head-only electrical stunning showed an increase in blood glucose lactate and hematocrit in the exsanguinated blood and a decrease in blood pH for the CO2 stunned Pietrain LY crosses, indicating that CO2 may compromise animal welfare (Becerril-Herrera et al., 2009). However, the fact that this concentration is below the required concentration in the EU (Council Regulation (EC) No. 1099/2009) should be taken into account.

Pre-slaughter stress resulted in an increase in blood temperature at exsanguination in a slaughterhouse using electrical stunning, although this relationship was not found in a slaughterhouse using CO2 stunning. The difference may be due to the stunning method (Hambrecht et al., 2004). In both slaughterhouses, the loin temperatures 30 min after slaughter were significantly higher in stressed pigs, although the difference was greater in the slaughterhouse with electrical stunning (Hambrecht et al., 2004). Also a comparison of anaesthesia, electrical stunning, CO2 stunning and captive bolt pistol showed a higher metabolic turnover rate after electrical stunning compared with CO2 stunning, indicating more stress in the electrically stunned pigs (Bertram, Stedkilde-Jørgensen, Karlsson, & Andersen, 2002). The difference between the two stunning methods is not straightforward, since they might compromise animal welfare in different ways and are therefore difficult to compare.

Furthermore, different levels of exsanguination CPK (creatine phosphokinase) and LDH (lactate dehydrogenase) were found in two different slaughterhouses, suggesting an effect of beef expertise (Weeding, Hunter, Guise, & Penny, 1993). One slaughterhouse had two types of electrical stunning, while the other slaughterhouse had both CO2 stunning and electrical stunning, but the difference in CPK and LDH values between the two slaughterhouses was independent of the stunning system, indicating that it was an effect of the pre-stunning treatment and not of the stunning system.

The development of the group stunning system using CO2 has reduced pre-slaughter stress by eliminating the use of electric prods and the restraining and isolation procedures. As described in Section 7 (“Race to the stunning chamber”), it has been shown that the stress before stunning has been reduced compared with single chute gas stunning (Steier et al., 2001). This system therefore has many advantages with regard to handling prior to stunning.

Animal welfare during stunning can therefore be challenged in two ways – the stunning process itself (pain and fear), and the effectiveness of the stunning process to ensure that the animal is unconscious at stunning. The fear and pain can be measured indirectly using the blood parameters as described in the other parts of the review as related to stress, e.g. lactate. The degree of unconsciousness can be measured by clinical examination such as corneal reflex and nose pricks (Nowak et al., 2007; Velarde, Gispert, Faustiano, Diestre, et al., 2000). In the WQ®, stunning effectiveness is measured by assessing the corneal reflex, righting reflex, rhythmic breathing and vocalizations.

In summary, the welfare of finishing pigs at stunning can be measured by blood glucose, lactate and hematocrit, as well as blood temperature. This could be combined with a continuous measurement of the CO2 concentration in the stunning system and by measuring aversive reactions towards CO2. In addition, vocalizations may be a good indicator of welfare when pigs are driven into the stunning chamber or are restrained in connection with stunning.

9. Sticking

Inadequate sticking constitutes a potential welfare issue when the bleeding takes place too slowly and there is a risk that the pigs may show signs of recovery during bleeding. The length of the sticking wound has been shown to significantly affect the rate of blood loss, with a long sticking wound resulting in faster exsanguination (Anil, Whittington, & McKinstry, 2000). The study used head-only electrical stunning, and one of the challenges reported is convulsions (Anil et al., 2000) that may render a proper sticking wound difficult to perform. A German survey has shown that, of 2,707 animals examined, 1.1% showed signs of regaining consciousness and sensibility three minutes after sticking (Troeger, Moje, & Schurr, 2005). If this is not detected, and the pig is subjected to sticking, it might be scalped alive. It is assumed that this must be extremely painful for the pigs and that it compromises the welfare of the pig, as described in Table 1. Measuring the quality of the sticking process is therefore crucial to the welfare of the animal.

In summary, if the sticking process is not performed efficiently, the pigs can regain consciousness and thereby experience great pain. A method for measuring sticking efficiency could be the vision-based system described below.

10. General discussion

As described in the previous sections, animal welfare on the day of slaughter can be compromised in different ways, including hunger and thirst, and fear (e.g. new environment, mixing of animals) and pain (e.g. skin damage during driving and stunning). There is still room for discussion of the relative importance of the different stressors and how to measure and thereby document welfare as required by legislation. An overview of the main stressors and suggested measurements is given in Table 5. Several of the measurements in the table are not specific to one stage of the day of slaughter, but are rather more general to this kind of stressor.

The blood concentration of glucose, lactate and the creatine kinase activity at exsanguination are promising as indicators of stress in general on the day of slaughter. In particular, a close relationship is seen between the level of the metabolites and incidents just before stunning (Brandt et al., 2013). However, when interpreting the values, other factors, such as lairage time, need to be taken into consideration.
serum concentration of CK increases and reaches its peak 6 h after a stressful situation and returns to basal level 48 h later (Anderson, 2010, cited from Correa et al., 2013). In comparison, plasma lactate increases to its maximum level within four minutes of physical exercise and returns to basal level in 2 h (Anderson, 2010, cited from Correa et al., 2013). Therefore, CK measured in the exsanguinated blood can be used as an indicator of events that most probably take place the whole way from the farm until sticking, even with long lairage times, while lactate, in particular, can be used as an indicator of events taking place closer to slaughter.

Skin damage is a very clear indicator of both aggression and pain due to inadequate equipment at the slaughterhouse. Skin damage is more easily assessed on the carcass than on the live pig (Barton Gade et al., 1996) and might in the future be automatized using a vision based technique. However, skin damage correlates with the blood concentration of CK, and this might be a better candidate for an online measurement of animal welfare, since there is a greater possibility of it being automated.

Cortisol has often been used as a measurement of stress. Saliva samples for cortisol analysis can be collected by getting the pigs to chew on cotton swabs (Peeters et al., 2008; Smulders, Verbeke, Mormède, & Geers, 2006). However, it has been argued that the method is somewhat inaccurate (Mormède et al., 2007). The secretion of cortisol follows a diurnal pattern and is pulsatile with a periodicity of approximately 90 min (Mormède et al., 2007), which needs to be taken into consideration when measuring cortisol. Peaks in cortisol concentration occur approximately 20 min after a stressful event, and therefore the cortisol concentration found in blood samples collected at exsanguination may reflect events taking place earlier in the handling process than the stunning (Warriess et al., 1994). The advantage, however, is that the method is relatively non-invasive. Urine samples provide a sample that has accumulated over several hours, thereby compensating for the fluctuations found in plasma samples of glucocorticoids (Mormède et al., 2007), and are therefore more representative of a general stress level rather than stress related to single events. Furthermore, the sampling can be performed non-invasively (Mormède et al., 2007). Rabaste et al. (2007) collected urine samples on the slaughter line for cortisol analyses. Urine sampling and analysis could maybe be automatized in the future.

Animal vocalizations indicate specific emotional states (Boissy et al., 2007) and are therefore highly relevant as an indicator for monitoring animal welfare. Sound can be used as a non-invasive method for measuring animal welfare given the development of unambiguous relations between the state of welfare and specific vocalizations (Manteuffel, Puppe, & Schön, 2004). The rate and duration of specific vocalizations can be used for welfare assessment (Manteuffel et al., 2004). STREMODO (STRESS and Documentation System) was developed for continuous monitoring of vocalizations in real-time (Schön, Puppe, & Manteuffel, 2004), and vocalizations might be the most promising indicator of the emotional state of pigs on the day of slaughter. An attempt has been made to discriminate between different types of vocalizations (Stöier, Sell, Christensen, Blaabjerg, & Aaslyng, 2011), but no effective systems currently exist for differentiating between different vocalizations, and it is recommended to record the number of vocalizations (Grandin, 2010). It is possible to measure and discriminate between pig vocalizations, although the interpretation requires simultaneous behavioral or physiological measurements and is not implementable based on current knowledge (Manteuffel et al., 2004; Schön et al., 2004; Stöier et al., 2011). A vision-based system – VisStick® – monitors the pigs after sticking and gives an alarm if no blood dripping from the pig’s snout is registered. In approval tests of five typical installations, a detection range of 98 to 100% of unstuck pigs was achieved. False positive results occurred in a range from 0 to 0.64% (Borggaard, Claudi-Magnussen, Madsen, & Stöier, 2011). Furthermore, spraying hot water (60 °C) on the faces of the pigs has been used to ascertain deaths combined with behavioral and clinical observations (Arnold et al., 2014; Troeger & Meiler, 2006). Provided that a robust vision system for detection can be developed, the method can probably be automated.

### Table 5

A summary of measurements of welfare of finishing pigs from the pick-up facilities until sticking.

<table>
<thead>
<tr>
<th>Checkpoint</th>
<th>Measurement</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pick-up facility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good housing on farm</td>
<td>↓ Haptoglobin</td>
<td>Geers et al. (2003)</td>
</tr>
<tr>
<td>† Mixing of unfamiliar pigs</td>
<td>↑ Skin damage</td>
<td>Barton Gade (2008)</td>
</tr>
<tr>
<td>† Aggression</td>
<td>↑ Skin damage</td>
<td>Geverink et al. (1996)</td>
</tr>
<tr>
<td>† Dehydration</td>
<td>↑ Albumin</td>
<td>Knowles and Warriss (2000)</td>
</tr>
<tr>
<td>† Mixing of unfamiliar pigs</td>
<td>↓ Salivary cortisol</td>
<td>Coutellier et al. (2007)</td>
</tr>
<tr>
<td>Loading</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use of electric prod</td>
<td>↑ Falls, ↑ vocalizations, ↑ lactate, ↑ heart rate</td>
<td>Correa et al. (2010)</td>
</tr>
<tr>
<td></td>
<td>↑ Albumin</td>
<td></td>
</tr>
<tr>
<td>Transport</td>
<td></td>
<td></td>
</tr>
<tr>
<td>† Distance and temperature</td>
<td>↑ Mortality</td>
<td>Barton Gade et al. (2007)</td>
</tr>
<tr>
<td>† Distance and mixing</td>
<td>↑ Mortality</td>
<td>Gosalvez et al. (2006)</td>
</tr>
<tr>
<td>† Temperature</td>
<td>↑ Mortality</td>
<td>Christensen and Jonsson (2007)</td>
</tr>
<tr>
<td>† Journey duration (0–4 h)</td>
<td>↑ Mortality</td>
<td>Sutherland et al. (2009)</td>
</tr>
<tr>
<td>† Stocking density</td>
<td>↑ Mortality</td>
<td>Warriss (1998)</td>
</tr>
<tr>
<td></td>
<td>↓ CK, ↓ lactate</td>
<td>Barton Gade and Christensen (1998)</td>
</tr>
<tr>
<td>Transport</td>
<td>↑ Serum CPK, ↑ LDH</td>
<td>Averso et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>↑ total protein</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↑ albumin, ↑ cortisol</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↑ Plasma glucose, ↑ lactate, ↑ albumin, ↑ CK</td>
<td>Mota-Rojas et al. (2009)</td>
</tr>
<tr>
<td>2 h transport</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unloading</td>
<td>↓ pH4</td>
<td>Van de Perre et al. (2010)</td>
</tr>
<tr>
<td>Lairage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-hour rest in lairage</td>
<td>↓ Glucose, ↓ lactate, ↓ albumin, ↓ blood temp, ↓</td>
<td>Mota-Rojas et al. (2009)</td>
</tr>
<tr>
<td></td>
<td>temp&lt;sub&gt;45&lt;/sub&gt;, ↑ pH&lt;sub&gt;4&lt;/sub&gt;, ↑ CK</td>
<td></td>
</tr>
<tr>
<td>† Lairage duration, ↑ stock density</td>
<td>↑ Skin damage</td>
<td>Geverink et al. (1996),</td>
</tr>
<tr>
<td>24 h rest</td>
<td>CK, ↑ cortisol</td>
<td>Smiecinska et al. (2011)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>† Vocalization, ↑ jamming, ↑ backing up</td>
<td>↑ Lactate</td>
<td>Edwards et al. (2010a)</td>
</tr>
<tr>
<td>Use of electric prod</td>
<td>↑ Cortisol, ↑ lactate, ↑ pH&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Hambrecht et al. (2004)</td>
</tr>
<tr>
<td>Sticking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>† CO&lt;sub&gt;2&lt;/sub&gt; concentration</td>
<td>↑ Corneal reflexes, ↑ lactate, ↑ pH&lt;sub&gt;4&lt;/sub&gt;, ↑</td>
<td>Nowak et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>↑ Vocalizations</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↑ Behavioral aversion</td>
<td>Warriss et al. (1994)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Velarde et al. (2007)</td>
</tr>
<tr>
<td>Sticking</td>
<td>↑ Sticking efficiency</td>
<td>Anil et al. (2000)</td>
</tr>
</tbody>
</table>
measurable indicators of animal welfare is the fact that they require the establishment of limits for good, acceptable and unacceptable levels of welfare.

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References


6. General discussion

The overall aim of this thesis was to add knowledge to the gap in the assessment of welfare of finishing pigs from farm to slaughter under commercial conditions by developing a protocol and an animal welfare index (AWI) for individual pigs. In addition, the aim was to investigate the relationship between individual behavioural or clinical welfare measurements as well as AWIs and physiological post-mortem measurements. The perspectives are to use selected post-mortem measurements as welfare approximations for future automation for documentation of welfare. This chapter discusses the methods and results from the observational studies presented in PaperI, PaperII and PaperIII, as well as the state of the art outlined in PaperIV in relation to the thesis aims and hypothesis. The chapter is split in two sections corresponding to the two partial aims of the thesis.

6.1 Development of an animal welfare assessment protocol from farm to slaughter (FTS protocol) and aggregation of the measurements into an animal welfare index (AWI)

The first partial aim was to develop a welfare assessment protocol for individual finishing pigs from farm to slaughter (PaperI and PaperII) and to aggregate the involved welfare measurements into an AWI for each of 6 stages (from the pick-up pen to the race) and an overall index including all 6 stages by use of expert opinion (PaperII). To fulfil these aims, the protocol was developed based on existing knowledge from the scientific literature. Secondly, the AWI was developed by inviting an expert panel to assign weights to the individual welfare measurements and to the different stages under the hypothesis: “Different animal welfare measurements within stage, as well as the different stages from farm to slaughter, will be assigned different weights in an aggregated welfare assessment”.

6.1.1 Development of a welfare assessment protocol

The selection of measurements for an animal welfare assessment protocol should meet criteria of validity, reliability and feasibility. Many indicators might be relevant to include in an operational welfare assessment system. One step of the recruitment procedure deals with identifying measurements of high relevance for animal welfare, as well as the inclusion of measurements, which, based on the multi-dimensional characteristics of the term animal welfare, may supplement each other (e.g. measurements of a high marginal validity). Furthermore, for assessment protocols focusing on implementation in commercial settings,
in the present thesis, feasibility and costs are other highly important quality parameters when selecting measurements relevant for an animal welfare assessment protocol (Rousing et al., 2001). In practise, selection of indicators for a welfare assessment system is based on weighing validity of measurement candidates against their reliability, operationality as well as feasibility. As an example, the present studies included both skin damage and aggressive behaviour as indicators of ‘absence of injuries’ and ‘expression of social behaviours’. Both measurements were considered highly valid for these criteria. In order to meet the criterion of feasibility, selecting just one of them was considered. According to the WQ®, measurements of social behaviour are more time consuming than the clinical measurements (Welfare Quality®, 2009). However, in the present work, the two types of measurements were not considered independent and able to stand alone, i.e. the marginal value was reduced, since scoring of aggressive behaviour cannot replace recordings of skin damage, because skin damage may occur from other causes than fighting, such as sharp edges on the housing equipment, mounting from other pigs and operator-inflicted bruises (Faucitano, 2001). On the other hand, aggressive behaviour may be expressed as threats, which may not result in visible skin damage of receiving pigs (Turner et al., 2006). Since the overlap between the two measurements was not complete as regards validity, both measurements were included in the protocol underlying the present thesis, which to some extent overruled the feasibility criterion for the inclusion of these measurements.

Another important quality parameter to consider is the reliability of the measurement i.e. reproducibility (such as between observer variation) and repeatability (within observer variation). In the present studies direct observation by more than one observer was necessary in lairage, why the use of the ethogram was discussed thoroughly in order to obtain high agreement between observes. Contrary, at loading, unloading and in the race, video recordings were used and one observer performed all video analyses. After finalising the analyses of all the video sequences, the observations and scores were verified by a second observation of a subsample corresponding to one half of the dataset.

### 6.1.2 Aggregation of measurements

The list of animal welfare measurements constitutes the animal welfare indicator protocol. In order to assess the level of animal welfare, expert opinion was used. Expert opinion is a common method to assign weights for animal welfare measurements for an overall welfare assessment e.g. at herd level (Burow et al., 2013; Jensen et al., 2012; Rousing et al., 2007).
According to Spoolder et al. (2003) a certain degree of subjectivity is unavoidable as regards choice of measurements as well as the relative weighting of measurements. Access to a stage index as well as an overall index allowed investigation of the possible relationships between the indexes and the physiological post-mortem measurements in order to identify possible candidate measurements for systematic welfare documentation (PaperIII). In order to be able to improve the level of welfare, the comparison with individual measurements is necessary (PaperI).

Typically, conduction of studies under commercial conditions increases the risk of missing values. For calculation of the index in PaperII, the average of the measurement across pigs was used to replace missing values, whereas in PaperIII, pigs with missing values were excluded. This different approach within the two papers underlying this thesis was based on the fact that in PaperII, 45 pigs were included in the calculations, which would have been reduced to include only 14 pigs for the overall index, if all pigs with missing values had been excluded. Furthermore, the aim of PaperII was to develop the index, not to conclude on the level of welfare. For PaperIII, the data set was largely reduced for the overall index as well (103 pigs), why the underlying basis was a large number of pigs (480). The index was calculated including the true values and not using averages since the aim was to detect relationships between the AWI and the post-mortem measurements.

In order to aggregate the measurements, the weighted linear sum of prevalence was used, as suggested by Burow et al. (2013). As mentioned, in WQ® indirect questioning resulted in non-linear weighting of grades, e.g. 20% lame pigs was not scored double as severe as 10% lame pigs (Welfare Quality®, 2009). The linear sum of prevalence used in the present thesis, means that if a pig is e.g. slipping and falling, the aggregated welfare assessment is the sum of those two measurements. Hence, the same index level within each stage could result from different welfare measurements, which to some extent were accounted for by multiplying the measurements by the expert weights. Thus, when two measurements were assigned the same weight by the expert panel, e.g. overlapping and moved by gate, two incidences were exactly twice as bad as one, constituting a different approach than used in WQ®. The present approach was chosen for transparency reasons. Transparency may help to increase the credibility, which is important in connection with documentation.

**Measurement weights using expert opinion**

Experts were asked to determine the importance of the measurements and stages involved in Study 2. The results presented in PaperII showed that the weights, assigned to the individual
welfare measurements by a panel of 38 experts differed within stage. No difference between stage weights was found. In general, there was good agreement among different expert affiliations, why the use of the average is considered reasonable. However, even if an expert panel agrees on the weightings of measurements for an aggregated assessment, there is still no guarantee, that the results reflect the true state of the animals.

Alternatively, aggregation could have been done assigning equal weights to all measurements. In fact, one expert assigned equal weights to all measurements and thus ascribed all measurements equal importance for welfare. From the WQ® project, it is known that experts assign different weights to different welfare measurements (Welfare Quality®, 2009), which 38 of 39 experts also did in the present study.

A drawback of using an expert panel is that the answers are specific for that particular panel. Including a larger number of experts, as in the present study, will however, provide larger certainty of the results. In WQ®, 5-6 animal scientists were involved in the selection of measurements, which may be considered a narrow panel of opinion, and it has been suggested that more experts should be involved for recalibration of the system at a later stage (Botreau et al., 2008). In WQ®, for the aggregation step from principles to an overall score, the opinion of other stakeholder groups was taken into account (Botreau et al., 2008). Composing a panel of foreign experts or including other stakeholder groups such as consumers and NGOs in the present study, would most likely have resulted in different weighting of the measurements. It has been suggested that other stakeholder groups such as laymen and consumers attach more importance to naturalness than e.g. biological fitness (Sørensen and Fraser, 2010). The wish for naturalness, e.g. access to outdoor areas, is impossible to meet in the period from farm to slaughter, why the choice of animal science experts only, was prioritized. However, in future research, other stakeholder groups could be included to gain knowledge of their opinion in relation to this delimited period in order to further develop and refine the index aiming at broader acceptance of the assessment.

6.2 Post-mortem physiological measurements as possible indicators of welfare measured by the AWIs

The second partial aim was to investigate the relationship between physiological post-mortem measurements and individual behavioural or clinical welfare measurements (PaperI) as well as the AWIs and based on these relationships to suggest possible candidate measurements for future automation for documentation of welfare (PaperIII). For addressing this aim, these hypotheses were tested: “There is a relationship between selected ante- and post-mortem
measurement obtained from pigs from farm to slaughter” (PaperI) and “There is a relationship between an AWI for each of 6 stages (AWI_{Stage}) as well as an overall index (AWI_{Overall}) obtained from pigs in the period from farm to slaughter and selected physiological post-mortem measurements under commercial conditions” (PaperIII).

The present study is among the first to investigate whether an animal welfare index can be expressed by selected physiological post-mortem measurements. The physiological measurements were selected on the basis of established knowledge such as the relationship between fatigue and lactate as well as between skin damage and CK (Gispert et al., 2000). Till now this knowledge comes from investigations of paired relationships using a subset of the day of slaughter e.g. transport or of selected handlings e.g. use of electric prods. The novel approach in this study was to integrate several physiological post-mortem measurements with the AWI for the entire day of slaughter, obtained from an observational study.

Physiological measurements obtained from blood samples such as CK and lactate were included in the studies although these are not directly implementable at the moment, as no analytical technique for on-line measurements at the slaughter line exists. However, if some of the measurements were identified as key parameters for documentation of welfare, it might act as a potential for future development of the needed methodology. The method needs to comply with slaughter line speed of 800 pigs per hour. The lactate scout analyser is a useful tool to measure real-time lactate concentrations in exsanguination blood at commercial abattoirs (Rocha et al., 2015) for research purposes, however, the method is too expensive regarding labour costs under Danish commercial conditions, why on-line automatic measurements are needed.

Below, the selected physiological post-mortem measurements are discussed in relation to the ante-mortem observations and their suitability as possible candidates for automatic measurements in future systematic monitoring of welfare. Furthermore, one method of identifying levels of welfare above vs. below a certain threshold is suggested.

**Lactate and glucose**

The plasma concentration of lactate was positively (but not statistically significant, PaperI) correlated to the occurrence of slipping, falling, overlapping and being moved by the gate in the race to stunning. However, when summing the measurements (the occurrence of slipping, falling, overlapping and being moved by the gate in the race), a significant relationship between the simple sum of measurements and plasma concentrations of glucose and lactate
was found (Brandt et al., 2012, appendix 10.3). In PaperIII, the result was confirmed to some extent as a weak but statistically significant relationship was found between lactate and AWI_{Race}. Previous studies found significant relationships between behavioural observations immediately prior to stunning and exsanguination blood lactate (Correa et al., 2010; Edwards et al., 2011; Edwards et al., 2010a; Hambrecht et al., 2004). In addition, Edwards et al. (2010a) found that pigs experiencing both jamming and backing up had a greater lactate concentration compared with pigs that did not experience any of these events. Since plasma lactate increases to its maximum within four minutes of physical exercise and returns to basal level in two hours (Anderson 2010, cited from Correa et al., 2013), the level measured at exsanguination reflects events experienced within up to 2 hours prior to stunning. Hence, the plasma concentration of lactate in exsanguination blood may be a potential candidate for online assessment of welfare related to the occurrence of specific behaviours (the occurrence of slipping, falling, overlapping and being moved by the gate) prior to slaughter. Other measurements need to be included to cover the entire period from farm to slaughter, which usually lasts longer than two hours. The finding of a significant positive correlation between plasma lactate and whole blood lactate concentrations (Brandt et al., 2014, appendix 10.3, Edwards et al., 2010c) facilitates automation of the quantification of lactate, making it more feasible as a candidate.

Based on the findings of correlations between plasma concentration of lactate and behaviour in the hours up to sticking, a preliminary investigation on identifying levels of welfare measured by AWIs above vs. below a certain threshold was suggested using AWI_{Race} and lactate as an example (PaperIII). In a future operational welfare assessment, the establishment of cut-off values is necessary to reach unambiguous conclusions, and more research is needed to determine the absolute levels of acceptable and unacceptable welfare of finishing pigs from farm to slaughter. The exsanguination blood lactate is dependent on stunning method, as Hambrecht et al. (2004) found different levels of lactate between CO\textsubscript{2} and electrical stunning in two different abattoirs. Hence, these cut-off values will need to be specific for each stunning method. In future studies, expert opinion may be used to determine such cut-off values, as in the WQ® Project, where four categories of acceptability was established; Excellent welfare, Enhanced welfare, Acceptable welfare and Not classified (~unacceptable) (Botreau et al., 2009; Veissier et al., 2011).

The results presented in PaperI showed that the plasma concentration of glucose was statistically significantly positively correlated to the occurrence of slipping and being moved by the gate in the race to stunning and a significant relationship was found between the
plasma concentration of glucose and the AWI during transport (negative) and between glucose and unloading (positive) (PaperIII). Furthermore a statistically significant negative relationship was found between the plasma concentration of glucose and the AWI_{Overall}. The bidirectional results could be because glucose was measured in the blood and not in the meat. The concentration of glucose in the blood is not only influenced by uptake of glucose from the feed and hence time since last feeding, but also by degradation of glycogen. Both factors may increase the blood concentration, but also increase the uptake into the muscles due to a need for energy, which then will decrease the concentration of glucose in the blood (Cunningham, 2002; Pearson and Young, 1989). In Study 1 of the present thesis, positive correlations were seen between glucose and the occurrence of slipping and being moved by the gate in the race, but these results were not confirmed in Study 2 where the relationship between the plasma concentration of glucose and AWI_{Transport} and AWI_{Unloading}, respectively, pointed in different directions. Increased concentration of plasma glucose can be stress-induced (Knowles and Warriss, 2000). However, when physical activity becomes strenuous, glucose decrease and lactate increase (Knowles and Warriss, 2000). The positive correlations between glucose and events in the race as found in Study 1, could therefore indicate a stress response immediately prior to slaughter. Further, the negative relationship between glucose and the AWI in the race in Study 2 could indicate that pigs were fatigued immediately prior to slaughter.

Taken together the results of the two studies regarding the plasma concentration of glucose as a possible indicator of welfare warrants further studies, and preferably under conditions, where factors such as fasting time and duration of lairage can be controlled for. At present, the results suggest that plasma concentration of glucose is not an unambiguous candidate for the monitoring of animal welfare from farm to slaughter, when only the exsanguination blood sample is available.

Previous studies on plasma concentration of glucose in relation to pre-slaughter handling, examined e.g. transport duration (Becerril-Herrera et al., 2010; Mota-Rojas et al., 2012) and lairage duration (Mota-Rojas et al., 2009), effect of sex and season (Averos et al., 2007), but none of the studies including glucose has done behavioural or clinical assessments and thus cannot be related directly to the results of this thesis. Furthermore, factors such as lairage duration (Mota-Rojas et al., 2009) and time of last feeding may influence the whole blood concentration of glucose (Cunningham, 2002) and lactate; information which will remain unknown in future surveillance system based on exsanguination blood analyses. Future studies could examine glucose and lactate measured continuously in the period from farm to
slaughter in order to identify their mutual relationship and elucidate whether lactate as a measurement of fatigue could be sufficient for online monitoring.

**Creatine kinase (CK)**

In contrast to the plasma concentration of glucose and lactate, information on feeding etc. is not necessary for interpretation of the creatine kinase activity, which is one of the potential physiological candidate measurements. CK measured in the exsanguination plasma correlated positively to skin damage score on the shoulder assessed on the slaughter line (PaperI) and skin damage score assessed in the pick-up pen, after lairage and post-mortem (PaperIII). These findings were expected, since CK is known to increase in humans as a result of tissue damage or muscular effort (Brancaccio et al., 2007). In addition, a statistically significant positive relationship between CK and the AWI\textsubscript{Unload} as well as a tendency for a positive relationship between CK and AWI\textsubscript{Overall} was found (PaperIII).

Since the serum concentration of CK increases and reaches its peak six hours after a stressful situation and then returns to basal level within the next 48 hours (Anderson 2010, cited from Correa et al., 2013) the relationship with the AWI\textsubscript{Overall} seems to be of higher relevance for the aim of this thesis than the other relationships. Earlier studies have reported increased CK in pigs after transport or transport-related stressors. Mota-Rojas et al. (2009) found significantly increased CK values after 118 km of transport compared to baseline values. In addition, Averos et al. (2007) found that CPK (creatinephosphokinase) increased significantly after unloading compared to before loading, and that the level was slightly decreased in the exsanguination blood compared to after unloading. Furthermore, Correa et al. (2013) found increased exsanguination CPK in pigs transported by potbelly trailers compared to double-deck lorries. However, Correa et al. (2010) did not find differences in CK between pigs loaded by use of electric prods compared to board and paddle. Taken together, CK seems to be sensitive to different stressors and probably includes responses to stressors occurring earlier in the period from farm to slaughter than what may be picked up by the plasma concentration of lactate. This could be stressors occurring on farm if the total duration of transport and lairage is less than six hours or reflect events happening during the entire period from farm to slaughter.

As mentioned, statistically significant positive correlations between CK and skin damages were found. Since skin damage is related to pain, and thus directly to animal welfare including the affective state of the animals, it is important to include this measurement in a welfare assessment. Today skin damage is included in the veterinary inspection at Danish
slaughter lines. However, an on-line automatic monitoring of skin damages could be considered an advantage in terms of time used as well as to optimize standardization. Since CK is characterised by being a valid indicator of skin damage, as found in the present studies, as well as in a previous study (Barton, 2008), the CK activity in exsanguination blood may be a promising candidate for future on-line documentation mainly related to skin damages. Thus, CK may, in combination with other potential candidates, be relevant for future automation of documentation of welfare of pigs from farm to slaughter.

**Albumin and total protein**

The plasma concentration of albumin and total proteins in the exsanguination blood were added to Study 2 in order to include measurements of dehydration (Knowles and Warriss, 2000), which is an important part of animal welfare, and covers several definitions of animal welfare including animal functioning and affective states (thirst).

The results of Paper III showed a statistically significant positive relationship between AWI\textsubscript{Race} and plasma concentration of albumin at exsanguination. Albumin and total protein quantified at this point are probably not specific measurements for a particular stage in the period from farm to slaughter, but more related to the interval since the pigs had access to water. The result found for albumin and AWI\textsubscript{Race} could, however, be due to linked effects, e.g. a dehydrated pig may find it more difficult to cope with the automatic gate in the race to stunning, than a hydrated counterpart. A previous study found increased serum albumin and total protein when comparing sampling after unloading from transport with sampling prior to loading onto a lorry and an intermediate value at exsanguination (Averos et al., 2007). Both measurements are thus possible indicators of dehydration in pigs during transport. However, in the study by Averos et al. (2007), season did not influence serum albumin, while total protein was higher in summer than in winter, indicating that total protein may be dependent on ambient temperature, and that knowledge of the plasma concentration of albumin and total protein obtained at exsanguination does probably not provide similar information about the conditions of the animals.

In contrast to albumin, which was related to AWI\textsubscript{Race}, there was a significant relationship between total protein in the exsanguination blood and AWI\textsubscript{Pick-up} (positive), AWI\textsubscript{Loading} (positive) and AWI\textsubscript{Unloading} (negative). Hence, the two measurements did not show similar relationships with the AWIs (Study 2). According to Knowles and Warriss (2000), both measurements show the same pattern of response to dehydration. In Study 2, a correlation between the two measurements 0.53 was found, which could indicate different patterns of
change, possibly due to a dietary effect as suggested by Knowles and Warriss (2000). Future studies should measure total plasma protein and plasma albumin experimentally in order to identify causal relationships by exposing the animals to thirst and record the responses in the two possible indicators. When these relationships are known, the suitability of the measurements as candidates for on-line measuring of dehydration can be determined including the need to include one or both measurements.

**Blood temperature, muscle temperature and pH**

Until now, possible metabolic measurements of animal welfare have been discussed. However, it is also possible that other physiological characteristics such as blood and muscle temperature or muscle pH may be linked to animal welfare. As described in section 4.3, Temp45 was excluded from Study 2 due to weak correlations with the ante-mortem measurements found in Study 1. Similarly, no relationship between blood temperature and pH45 and the ante-mortem observations was found. Despite statistically significant positive correlations between pH22 and the occurrence of overlapping, being moved by the gate and falling in the race to stunning, this variable was excluded from Study 2 as well, since sampling of muscle pH causes a risk of damaging the meat. Hence, Study 2 included blood temperature and pH45. However, technical difficulties with the measuring equipment combined with the slaughter line speed, resulted in a high proportion of missing values, and a reduced dataset. Thus, blood temperature and pH45 was analysed in separate models. Sampling of post-mortem muscle pH causes a risk of damaging the meat and is thus not expected to become automated, due to practical considerations. However, in contrast to pH45, blood temperature may still be a candidate for on-line monitoring of welfare from farm to slaughter in pigs, since body temperature has been shown to be related to transport duration (Becerril-Herrera et al., 2010; Mota-Rojas et al., 2012) and handling prior to slaughter (Hambrecht et al., 2004). The development of automatic measuring equipment e.g. incorporated in the Rotastick®, may make it possible to comply with slaughter line speed. Hence, in future studies, automatic measurements of blood temperature should be used to confirm the relationship between pre-slaughter handling and blood temperature e.g. by exposing pigs to experimental stressors.

**Adrenocortical measurements of welfare in pigs in the period from farm to slaughter?**

Within the present thesis, focus has been on physiological correlates of physical conditions such as dehydration and fatigue as possible candidates for automated measurements of
welfare and has not included measurements of the adrenocortical activity or reactivity, although these measurements, particularly some decades ago, were regarded as valid measurements of animal welfare (Barnett and Hemsworth, 1990). Changes in the plasma concentration of cortisol can be used to reflect acute stress, and provided the availability of a baseline measurement, cortisol measured after exposure to a stressor could have been used as a measurement of the level of acute stress, since cortisol is known to increase as a result of e.g. restraining or acute pain (Moberg, 2000). However, interpretation of the plasma concentration of cortisol, measured e.g. prior to and after lairage, in terms of animal welfare, would not have been straightforward, since cortisol also increases as a result of non-aversive events such as play or mating (Moberg, 2000). The facts that cortisol secretion follows a diurnal pattern and is pulsatile with a periodicity of approximately 90 minutes (Mormède et al., 2007), means that the interpretation in terms of animal welfare is further difficult, when the exsanguination blood concentration is the only available measurement. Thus, analysis of the plasma concentration of cortisol was not included in the work presented in this thesis.

6.3 Considerations regarding choices of statistical analyses

In PaperI, the PCA was used to create a descriptive overview of the relationships between the included measurements. The first and second dimensions of the PCA plot, PC1 and PC2, explained 13% and 11% of the variance, respectively. To further describe the relationships between individual ante-mortem welfare measurements and physiological post-mortem measurements, the correlation coefficients were calculated for the most biologically relevant relationships determined from the PCA plot. No firm guidelines are available for the determination of the level of an influential loading in a PCA. Almost 20 years ago, a limit of 0.5 was suggested (Sharma, 1996). However, this suggestion is not described in recent literature (Næs et al., 2010b). As PCA is a descriptive method, which shows patterns in the data, no cut-off values were used in this study. Instead the correlation coefficients were calculated.

To further investigate the relationships between the welfare measurements and the physiological post-mortem measurements, the observed frequencies of the welfare measurements were summarized for each of four stages: loading, unloading, lairage and race to stunning. Using a linear mixed model, these simple sums of welfare measurements were analysed as fixed effects and the post-mortem plasma concentration of glucose, lactate and creatine kinase activity, as well as blood and loin temperature and pH as response variables. Herd was included as random effect. These results were presented as a conference paper (See
Chapter 10.3) and indicate that the summation of the welfare measurements was related to the physiological post-mortem measurements. However, the simple sum of welfare measurements does probably not represent the true welfare consequences for the pigs and paperII aims to provide a multi-dimensional animal welfare index (AWI) using a weighted sum of welfare measurements. PaperIII then provides a linear regression model including the weighted linear sum of welfare measurement prevalences (the AWIs). When testing several hypotheses (e.g. five post-mortem physiological blood measurements), the level of significance may either be lowered by Bonferroni correction (P=0.05/5=0.01) in order to avoid false associations, or conclusions should be drawn carefully.

In PaperIII, the results are presented as the regression coefficients (PaperIII, Table 8). In order to elaborate the adequacy of this approach, Figure 3 illustrates the predicted against the measured AWI values per stage. To further illustrate data, boxplots were created by subdividing the explanatory variables in three categories by splitting the difference between the minimum and the maximum value in three equal parts (only statistically significant relationships are shown). The plots (Figure 3) indicate similar results as presented in Table 8 of PaperIII.

Pick-up facility

![Pick-up facility](image-url)
Race to the stunning chamber

**Figure 3.** AWIs were calculated for finishing pigs on the day of slaughter. The plots show the predicted against the measured AWI values per stage. In addition, boxplots are shown, by subdividing the explanatory variables in three categories by splitting the difference between the minimum and the maximum value in three equal parts (e.g. for total protein: min value = 57.8 g/L, max value = 88.6 g/L, thus category 0 corresponds to intervals for total protein of 57.8 g/L to ≤ 68.1 g/L, category 1 to 68.1 g/L ≤ 78.3 g/L and category 2 to 78.3 to 88.6 g/L).

Although usable for provision of an overview of multivariate data, the PCA plot was not presented in PaperIII, due to the many missing values which would have increased the risk of biased interpretations. However, the PCA loading plot is shown in Figure 4 below. Blood temperature and pH was excluded from the analyses due to too many missing values. As illustrated in Figure 4, 35% of the variance was explained by the first two principal components. It should be taken into consideration that both the loadings and the explained variation is rather low, indicating that some patterns are present in the data, but that conclusions should be drawn carefully. Based on Figure 4, AWI\textsubscript{Loading} and total protein were related with respect to PC 2 (15 % of the variation) which is in agreement with Figure 3 and Table 8 in PaperIII indicating a relationship, although not strong. AWI\textsubscript{Unloading} is fairly close to glucose and in the same quadrant as logCK and negatively correlated to total protein (Figure
4) which corresponds to Figure 3 and Table 8 in PaperIII. In contrast, the AWIPick-up and total protein were in the same quadrant pointing in the same direction although far apart (Figure 4), even though there was a statistically significant relationship shown in Table 8, PaperIII. AWIRace and lactate could be correlated based on Figure 4 and the relationship was statistically significant as shown in Table 8 in PaperIII. However, based Figure 3 this relationship is due to a single observation and conclusions should be drawn carefully.

Thus, the suggested physiological post-mortem measurements may show potential as candidates for automatic documentation of animal welfare under the present conditions, but more research, including a larger variation in handling and other events known to challenge the welfare of the pigs, is needed before exact conclusions can be drawn.

**Figure 4.** Principal component analysis (PCA) of relationships between the weighted log-transformed animal welfare indexes, AWI (AWI_Pick-up, AWI_Loading, AWI_Transport, AWI_Unloading, AWI_Lairage, AWI_Race) and post-mortem measurements (exsanguination plasma glucose, lactate, albumin total protein and creatine kinase activity). The analysis was carried out including the 418 pigs where blood samples were analysed. Data was standardized by dividing with the standard deviation.
7. Conclusion and perspectives
The overall aim of this thesis was to add knowledge to the gap in the assessment of welfare of finishing pigs from farm to slaughter under commercial conditions, by developing a protocol and an animal welfare index (AWI) for individual pigs. In addition, the aim was to investigate the relationship between individual behavioural or clinical welfare measurements as well as the AWIs and physiological post-mortem measurements. The perspectives are to use selected post-mortem measurements as welfare approximations for future automation for documentation of welfare.

Based on two observational studies conducted at two Danish abattoirs as well as an online expert opinion survey, the work underlying this thesis has led to the establishment of a welfare assessment protocol for finishing pigs in the period from farm to slaughter and an aggregated animal welfare index (AWI) – both important tools for future research and welfare assessment.

Statistically significant, however relatively weak relationships were found between the individual welfare measurements and post-mortem measurements and between the AWIs and the post-mortem measurements, suggesting that, at this stage, exact conclusions cannot be drawn regarding possible physiological candidates for development of a future on-line tool for documentation of animal welfare on commercial abattoirs.

The weak relationships found in this study could be due to lack of variability in the study conditions, such as season, housing and transport conditions and number and handling of the pigs. The results of the present studies were based on a relatively small dataset and generally high level of animal welfare (low prevalence of measurements), and more research is needed including a larger variation in handling and other events known to challenge the welfare of pigs in order to support these relationships.

In order to incorporate automatic measurements (given the development of technological solutions), thresholds must be decided for acceptable and unacceptable AWI levels in order to determine limits for the physiological post-mortem measurement for documentation purposes. These limits may be established e.g. using expert opinions. An initial approach to examine whether the post-mortem measurements can be used to identify AWIs categorised as ‘below threshold’ has been suggested in this thesis, however, more research is needed to investigate the applicability of the physiological post-mortem measurements for the assessment of welfare of pigs from farm to slaughter.

Thus, taken together, the studies underlying this PhD thesis has added knowledge to the gap in the assessment of welfare of finishing pigs from farm to slaughter and created important
tools for future welfare assessment, as well as the basis for future research aiming to reach a feasible tool for documentation of animal welfare in the slaughtering industry.
8. Additional scientific activities

<table>
<thead>
<tr>
<th>Courses</th>
<th>ECTS</th>
<th></th>
</tr>
</thead>
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<tr>
<td>Business course for industrial PhD students</td>
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<tr>
<td>Crane seminar, The good the bad and the ugly side of human animal relations</td>
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<td>Animal ethics</td>
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<td>Applied statistics with R for the agricultural, life and veterinary sciences</td>
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<tr>
<td>Measuring and assessing animal welfare at herd level</td>
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<td>AU 2013</td>
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<tr>
<td>Interpretation of animal stress responses</td>
<td></td>
<td>AU 2013</td>
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<tr>
<td><strong>Total</strong></td>
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**Posters**


(Aaslyng, M.D., Brandt, P., Blaabjerg, L.O., Støier, S., 2013. Assessment and incidence of skin damage in slaughter pigs. Poster presentation at the ICoMST 2013.)


(Aaslyng, M.D., Blaabjerg, L.O., Brandt, P., 2012. Documentation of animal welfare of pigs on the day of slaughter. Poster presentation at the ICoMST 2012.)

**Additional peer reviewed**

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<th>Dissemination</th>
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<tr>
<td>Presentation of PhD-project at Axelborg at: &quot;Inspirationsdag vedrørende logistik, Arbejdstilrettelæggelse og flytning af grise”. 2012.</td>
</tr>
<tr>
<td>Presentation of PhD-project at AU for students taking “Applied Ethology”. 2012.</td>
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<tr>
<td>Presentation for students in “Råvarekvalitet” at KU. 2013.</td>
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<tr>
<td>Joint supervisor for a student writing a 7.5 ECTS assignment on “Assessment of skin damage scores on the day of slaughter”. The student was enrolled at Copenhagen University. 2012.</td>
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9. References


Smiecinska, K., Denaburski, J., Sobotka, W., 2011. Slaughter value, meat quality, creatine kinase activity and cortisol levels in the blood serum of growing-finishing pigs slaughtered immediately after transport and after a rest period. Polish Journal of Veterinary Sciences 14, 47-54.


10. Appendix

10.1 Welfare assessment protocol for finishing pigs from farm to slaughter (FTS)

<table>
<thead>
<tr>
<th>FTS measurement</th>
<th>Definition</th>
<th>Stage</th>
<th>Level of recording</th>
<th>Method of recording</th>
<th>WQ® criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transport conditions</td>
<td>Description of weather conditions</td>
<td>Trans</td>
<td>Gr</td>
<td>Direct</td>
<td>3</td>
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<td></td>
<td>Description of the route</td>
<td></td>
<td></td>
<td></td>
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<td>Duration</td>
<td>Pick-up, hours (&lt;2 or &gt;8)</td>
<td>Pick-up</td>
<td>Gr</td>
<td>Interview</td>
<td>3</td>
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<tr>
<td></td>
<td>Loading, min</td>
<td>Loading</td>
<td>Direct</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Transport, hours</td>
<td>Trans</td>
<td>Direct</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Break during transport, min</td>
<td>Trans</td>
<td>Direct</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unloading, min</td>
<td>Unloading</td>
<td>Direct</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lairage, hours</td>
<td>Lairage</td>
<td>Direct</td>
<td></td>
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<td>Temperature</td>
<td>Reading of outdoor temperature gauge in the car</td>
<td>Pick-up</td>
<td>Gr</td>
<td>Direct</td>
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<tr>
<td>Group dynamics</td>
<td>Mixing</td>
<td>Pick-up</td>
<td>Gr</td>
<td>Interview</td>
<td>3</td>
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<td></td>
<td></td>
<td>Lairage</td>
<td>Direct</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group size</td>
<td>No. of pigs per group</td>
<td>Home pen, pick-up</td>
<td>Gr</td>
<td>Direct</td>
<td>5</td>
</tr>
<tr>
<td>Stocking density</td>
<td>Measuring m² + count of pigs</td>
<td>Pick-up</td>
<td>Gr</td>
<td>Direct</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trans</td>
<td>Direct</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lairage</td>
<td>Direct</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feeding</td>
<td>Hours since last feeding</td>
<td>Home pen</td>
<td>Gr</td>
<td>Interview</td>
<td>1</td>
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<tr>
<td>Character of handling</td>
<td>0: the pig moved voluntarily or was driven by the herd,</td>
<td>Loading, unloading</td>
<td>Fo</td>
<td>Video</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>1: rattle stick or board (just touching),</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2: rattle stick, board or hand (single stroke),</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3: rattle stick, board or hand (repetitive strokes)</td>
<td></td>
<td></td>
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<td>Gate</td>
<td>Physically moved by the automatic gate</td>
<td>Race</td>
<td>Fo</td>
<td>Video</td>
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<tr>
<td>Measurement</td>
<td>Definition</td>
<td>Stage</td>
<td>Level of recording</td>
<td>Method of recording</td>
<td>WQ® criteria</td>
</tr>
<tr>
<td>----------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>----------------</td>
<td>--------------------</td>
<td>---------------------</td>
<td>--------------</td>
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<tr>
<td><strong>Pig behaviour</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Behaviour during handling</td>
<td>Reluctance to move, turning back</td>
<td>Loading, unloading</td>
<td>Fo</td>
<td>Video</td>
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<tr>
<td>Slipping</td>
<td>At least one of the pig’s legs slips, but the body does not come into</td>
<td>Loading, unloading, race</td>
<td>Fo</td>
<td>Video</td>
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<tr>
<td></td>
<td>contact with the floor</td>
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<td></td>
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<tr>
<td>Falling</td>
<td>The pig loses balance on one or more legs, and falls to its knees, or</td>
<td>Loading, unloading, race</td>
<td>Fo</td>
<td>Video</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>another part of the body other than the legs comes into contact with the</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>floor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aggression</td>
<td>Recorded as short bouts defined as &lt; 10 seconds and long bouts defined as</td>
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<td>Gr</td>
<td>Direct</td>
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<tr>
<td></td>
<td>≥ 10 seconds.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posture</td>
<td>Standing, sitting, lying</td>
<td>Lairage</td>
<td>Fo</td>
<td>Direct</td>
<td>3</td>
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<tr>
<td>Overlapping</td>
<td>Lifted by another pig</td>
<td>Loading, race</td>
<td>Fo</td>
<td>Video</td>
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<tr>
<td><strong>Clinical state of health</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Dead</td>
<td>Count</td>
<td>Unloading</td>
<td>Fo</td>
<td>Direct</td>
<td>7</td>
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<tr>
<td>Sick</td>
<td>Veterinary inspection. Pigs were isolated and humanely euthanized if</td>
<td>Unloading</td>
<td>Fo</td>
<td>Direct</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>required</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lameness</td>
<td>0: no lameness 1: uneven gait</td>
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<td>Fo</td>
<td>Direct</td>
<td>6</td>
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<tr>
<td>Hernia</td>
<td>Yes/no</td>
<td>Pick-up</td>
<td>Fo</td>
<td>Direct</td>
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<tr>
<td>Skin damage</td>
<td>Tail bite, yes/no Skin damage was assessed on 4 body parts: ear/head,</td>
<td>Home pen,</td>
<td>Fo</td>
<td>Direct</td>
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<tr>
<td></td>
<td>shoulder, middle and hindquarters on a 4-point scale: 0: None or minor</td>
<td>Lairage, PM</td>
<td></td>
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<tr>
<td></td>
<td>scratches, 1: few (&lt; 3) short (up to 3 cm) scratches, 2: &gt;3 short or ≥ 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>long scratch(es) (&gt; 3 cm), 3: ≥1 wound(s).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Measurement</td>
<td>Definition</td>
<td>Stage</td>
<td>Level of recording</td>
<td>Method of recording</td>
<td>WQ® criteria</td>
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<tr>
<td>-------------------</td>
<td>---------------------------------------------------------</td>
<td>-----------</td>
<td>--------------------</td>
<td>---------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Heart rate</td>
<td>Belts were fitted on selected pigs</td>
<td>Pick-up -→ sticking</td>
<td>Fo</td>
<td>Direct</td>
<td>3</td>
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<tr>
<td>CK</td>
<td>Blood sample collected at exsanguination</td>
<td>PM</td>
<td>Fo</td>
<td>Direct</td>
<td>6</td>
</tr>
<tr>
<td>Glucose, lactate</td>
<td>Blood sample collected at exsanguination</td>
<td>PM</td>
<td>Fo</td>
<td>Direct</td>
<td>(13)</td>
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<tr>
<td>Albumin, total protein</td>
<td>Blood sample collected at exsanguination</td>
<td>PM</td>
<td>Fo</td>
<td>Direct</td>
<td>(13)</td>
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<tr>
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<td>Fo</td>
<td>Direct</td>
<td>(13)</td>
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<tr>
<td>pH45</td>
<td>pH was measured 45 min after sticking in m. LD</td>
<td>PM</td>
<td>Fo</td>
<td>Direct</td>
<td>(13)</td>
</tr>
</tbody>
</table>

Gr = Group level, in the home pen where the focal pigs were marked for identification, an additional 50 pigs were marked with two lines on the back and the 90 pigs (40 focal + 50 additional) constitute the group.
Fo = Focal pig level
PM = post-mortem
### 10.2 Questionnaire

<table>
<thead>
<tr>
<th>Assessment of welfare of finishing pigs on the day of slaughter</th>
</tr>
</thead>
</table>

This questionnaire is about the welfare of finishing pigs on the day of slaughter. The aim is to develop an overall assessment of welfare, primarily based on behavioural observations. To fulfil this aim, we wish to grade the importance of various events during the day of slaughter for welfare of the pigs.

We will ask you to complete the questionnaire. The answers will be used anonymously, but eventually we would like to ask for your email address so we know who participated. When you answer the questions, it is under an assumption that the pigs are fit for transportation, and legislation is complied with. If you believe that factors other than those we have selected is important, we would like you to write them in the comments box.

#### 1. Education

*(Enter multiple answers)*

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<thead>
<tr>
<th>Agronomist</th>
<th>Veterinarian</th>
<th>Other</th>
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<tbody>
<tr>
<td>✔️</td>
<td>✔️</td>
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</tr>
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#### 2. Employed by

*(Enter only one answer)*

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<th>The University</th>
<th>The Authority</th>
<th>LF or DMRI</th>
<th>Consultancy</th>
<th>Abattoir</th>
<th>Other</th>
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<td>□</td>
<td>✔️</td>
<td>□</td>
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</tr>
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</table>
3. Pick-up pen

How important is it for me - when I need to assess pig welfare in the pick-up pen - to know whether and to what extent the pig has been / is exposed to the following five factors. Insert 1 x in each line where 1 is not important and 5 is crucial.

(Enter only one answer per question)

<table>
<thead>
<tr>
<th></th>
<th>1. Not important</th>
<th>2.</th>
<th>3.</th>
<th>4.</th>
<th>5. Crucial</th>
</tr>
</thead>
<tbody>
<tr>
<td>The pig has bruises and wounds - rated on a scale of 0-3 where: 0: none, 1: few scrapes, 2: many bruises, 3: wounds</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Duration</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Stocking density</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Mixing</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Fast</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The next page shows 4 video sequences. Watch them and assess:
How important is it for me - when I need to assess the pig welfare during loading - to know whether and to what extent the pig has been / is exposed to the factors described.

### 4. Loading

Insert 1 x in each line, where 1 is not important and 5 is crucial.

*(Enter only one answer per question)*

<table>
<thead>
<tr>
<th></th>
<th>1. Not important</th>
<th>2.</th>
<th>3.</th>
<th>4.</th>
<th>5. Crucial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of loading</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Handling score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The pig is reluctance to move</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The pig is turning back</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The pig is slipping</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The pig is falling</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The pig is overlapping</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5. Transport

How important is it for me - when I need to assess pig welfare during transport - to know whether and to what extent the pig has been / is exposed to the following four factors. Insert 1 x in each line, where 1 is not important and 5 is crucial.

(Enter only one answer per question)

<table>
<thead>
<tr>
<th>Factor</th>
<th>1. Not important</th>
<th>2.</th>
<th>3.</th>
<th>4.</th>
<th>5. Crucial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Driving style</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of transport</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of breaks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambient temperature at pig height</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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136
The next page shows three video sequences. Watch them and assess:
How important is it for me - when I need to assess pig welfare during unloading - to know whether and to what extent the pig has been / is exposed to the factors described.

### 6. Unloading

**Insert 1 x in each line, where 1 is not important and 5 is crucial.**

(Enter only one answer per question)

<table>
<thead>
<tr>
<th></th>
<th>1. Not important</th>
<th>2.</th>
<th>3.</th>
<th>4.</th>
<th>5. Crucial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of unloading</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Handling score (e.g. the pig moves voluntarily, is moved by use of paddle and/or board, or is receiving repetitive strokes)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>The pig is reluctant to move</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>The pig is turning back</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>The pig is slipping</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>The pig is falling</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Other</td>
<td>----------------</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>------------</td>
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<tr>
<td></td>
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<td></td>
<td>----------------</td>
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<td>----</td>
<td>------------</td>
</tr>
</tbody>
</table>
7. Lairage

How important is it for me - when I need to assess pig welfare - to know whether and to what extent the pig has been / is exposed to the following nine factors. Insert 1 x in each line, where 1 is not important and 5 is crucial.

(Enter only one answer per question)

<table>
<thead>
<tr>
<th>Factor</th>
<th>1. Not important</th>
<th>2.</th>
<th>3.</th>
<th>4.</th>
<th>5. Crucial</th>
</tr>
</thead>
<tbody>
<tr>
<td>The pig has bruises and wounds - rated on a scale of 0-3 where: 0: none, 1: few scrapes, 2: many bruises, 3: wounds</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of lairage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aggression, recorded as short (&lt; 10 sec) and long (&gt; 10 sec) bouts.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of posture changes (lying, sitting, standing)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The pig is mounting another pig</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The pig is being mounted by other pigs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency to the pig lie down</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of the time the pig is lying</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Other

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Race to stunning

The next page shows 3 video sequences. Watch them and assess:
How important is it for me - when I need to assess pig welfare in the race to stunning - to know whether and to what extent the pig has been / is exposed to the factors described.

8. Race to stunning

Insert 1 x in each line, where 1 is not important and 5 is crucial.
(Enter only one answer per question)

<table>
<thead>
<tr>
<th></th>
<th>1. Not important</th>
<th>2.</th>
<th>3.</th>
<th>4.</th>
<th>5. Crucial</th>
</tr>
</thead>
<tbody>
<tr>
<td>The pig is slipping</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The pig is falling</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The pig is overlapping other pigs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The pig is moved by the automatic gate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Other
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
On a standard day of slaughter
The purpose of this part of the questionnaire is to examine where in the chain from farm to slaughter pigs are exposed to the greatest strain. Therefore, an assessment of the impact of the different stages from the pick-up pen to the race to stunning is requested.

9. What impact has the following 6 stages on pig welfare on the day of slaughter? Insert 1 x in each line, where 1 is not important and 5 is crucial.

(Enter only one answer per question)

<table>
<thead>
<tr>
<th>Stage</th>
<th>1. Not important</th>
<th>2.</th>
<th>3.</th>
<th>4.</th>
<th>5. Crucial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pick-up pen</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Loading</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Transport</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Unloading</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Lairage</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Race to stunning</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

10. Email address


Thank you for your help.
10.3 Posters
Measuring welfare of finishing pigs from loading at the producer until sticking at a commercial abattoir

Pia Brandt¹,², Tine Rousing², Mette S. Herskin², Margit Dall Aaslyng¹

Introduction
The market and the consumers show increasing interest in and demands towards animal welfare. Documentation of animal welfare is mandatory at larger slaughterhouses from 2013 in the EU due to the regulation (1099/2009). Compliance with these requirements necessitates the development of tools for continuous monitoring of animal welfare. The aim was to evaluate different indicators of animal welfare to identify possible measures for future development of documentation of animal welfare on the day of slaughter.

Materials and Methods
Welfare indicators inspired by the Welfare Quality® Protocol (2009) were recorded.

Behavioral and management recordings included:
- Turning back
- Reluctance to move
- Slipping and falling
- Postures during lairage
- Overlapping in the driveway to stunning
- Driving

Post mortem recordings included:
- Collection of blood samples at sticking
- Blood temperature
- Assessment of skin damage
- Loin temperature and pH 45 min after sticking

In total 80 pigs from four producers were included. Correlations between the measured variables were calculated. For every event, i.e. loading, unloading, lairage and driveway to stunning, the recordings were summarized. Analyses of variance were performed to investigate the effect of the four events prior to slaughter on the post mortem blood concentration of glucose, lactate and creatine kinase activity (CK), blood and loin temperature and pH.

Results
There was a significant correlation between CK and skin damage score on the shoulder (P<0.0001) and between CK and slipping and falling in the driveway to stunning (P=0.03). Handling in the driveway to stunning affected the blood concentration of lactate (P=0.03) and glucose (P=0.005) significantly. There was no effect of handling in the driveway to stunning on CK. Further, there was a significant difference between producers on the concentration of lactate and CK. There were no significant effects of the behavior and management recordings on blood or loin temperature or pH.

Conclusion
The study indicates that the blood concentration of lactate, glucose and the creatine kinase activity are related to the behavior and management prior to slaughter and might be relevant indicators for documentation of animal welfare on the day of slaughter.

¹Danish Meat Research Institute, DK-4000 Roskilde, www.DMRI.com
²Department of Animal Science, Aarhus University, DK-8830 Tjele
Exsanguination blood lactate as an indicator of pre-slaughter welfare in finishing pigs

Pia Brandt,1,4 Tine Rousing,1 Mette S. Herskin,2 Margit D. Aaslyng2
1) Department of Animal Science, Aarhus University, DK-8830 Tjele, 2) Danish Meat Research Institute, DK-2830 Taarbæk, www.DMRI.com

INTRODUCTION
Pre-slaughter handling constitutes novel and potentially stressful experiences for pigs. The concentration of lactate in the exsanguination blood may indicate that finishing pigs have been exposed to stressful events prior to slaughter. The aim of the present study was to investigate the relationship between ante mortem observations and the plasma concentration of lactate (P-LAC) as part of an evaluation of welfare in finishing pigs at commercial abattoirs (Study 1). Furthermore, if lactate is to be used as an on-site indicator of pre-slaughter welfare at abattoirs, access to a fast analysis, such as whole blood analysis, is necessary. Hence, the secondary aim was to compare the concentration of lactate in whole blood (W-LAC) with P-LAC (Study 2).

STUDY 1
- 80 pigs from four herds were included.
- Behavioural and handling observations were carried out in the race to the stunning chamber and included slipping, falling, being moved by automatic gates and lifting by other pigs.
- Behavioural and handling measurements were recorded using one-zero sampling and summarized to scores 0 (no events observed), 1 (1 or more events) and 2 (2 or 3 events observed).
- At sticking, a blood sample was collected for analysis of P-LAC (automatic biochemistry analyser, ADVIA® Chemistry Systems, Siemens, Erlangen, Germany).

STUDY 2
- At sticking, blood samples from 107 pigs were collected.
- The blood samples were analyzed for W-LAC using a handheld lactate analyzer (Lactate ProTM Blood Lactate Test Meter, Arkray, Shiga, Japan) and P-LAC (automatic biochemistry analyser, ADVIA® Chemistry Systems, Siemens, Erlangen, Germany).

A significant relationship between the scores for behaviour and handling in the race and P-LAC was found (P = 0.008). Different letters (a and b) indicate a significant difference at P < 0.05. Thus, P-LAC might be an indicator of welfare of pigs at the abattoir.

Comparison of W-LAC and P-LAC showed a correlation of 0.90 suggesting that W-LAC can replace the more elaborate plasma analyses. One outlier has been removed.

CONCLUSION
- A significant relationship between handling and behaviour prior to slaughter and P-LAC, suggest that P-LAC may be used to indicate pre-slaughter welfare in finishing pigs.
- The faster W-LAC analysis is applicable.