PRESLAUGHTER TREATMENT OF PIGS

consequences for welfare and meat quality

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Les hommes ont oublié cette vérité, dit le renard. Mais tu ne dois pas l'oublier. Tu deviens responsable pour toujours de ce que tu as apprivoisé.

Antoine de Saint-Exupéry
Le petit prince

All the roads that lead you there are winding
All the lights that light the way are blinding

Oasis
Wonderwall

Listen as the syllables of slaughter cut with calm precision

Marillion
Assassin

Contents

Chapter 1

General Introduction

Fattening pig sector

In the Netherlands pork is the most popular meat, with 44.4 kg being eaten per head of the population each year (PVE, 1997). Of the total production, about 75% is exported. To maintain this production, the Netherlands have about 24.000 pig farms which are highly concentrated in the south of the country. Each year almost 20 million pigs are slaughtered in 32 slaughterhouses (Ministry ANF, 1996). In 1996, the human population was almost outnumbered by the number of pigs, but the outbreak of classic swine fever at the beginning of 1997 reduced the number of pigs with about 25%. One of the consequences of the publicity on the disease was that the attention was drawn once more to the environmental and welfare issues associated with modern pig production, which is characterized a.o. by indoor housing on slatted floors and no bedding, with a high number of animals per surface area. These intensive husbandry systems have been developed from the sixties onwards, thus having a rather short history compared with the long domestication history of the pig and requiring considerable physiological and behavioural adaptation.

The wild pig

The ancestors of domestic pigs are to be found among wild pigs of the species *Sus scrofa* which ranges throughout Eurasia (Epstein and Bichard, 1984). Wild pigs live mainly in dense brush or near dense cover. Away from civilization, they are found to be active mostly during the daylight (Graves, 1984). Pigs are omnivorous and spend a great deal of their time rooting in search for food. Pigs are social animals and wild pigs typically live in groups of two to five related females and their immature offspring (Graves, 1984). Other individuals may be loosely associated with this basic social unit, while adult wild boars tend to remain solitary. Piglets begin to form social dominance relationships with littermates within hours after birth. Because several females may combine litters, interactions with individuals from other litters begin very early in life (Graves, 1984).

Domestication

The earliest remains of domesticated pigs have been found in Turkey and date back to ca. 7000 B.C. (Reed, 1977). Later remains of pig bones have been found all over Eurasia, and domestic swine have been imported to most other continents. Several breeds have been developed and distributed during the latest centuries (Epstein and Bichard, 1984). In the process of domestication, the pig has changed in

morphology and physiology. Compared to the wild pig, the domestic pig with its round body form and less hairy skin has a high growth potential with an increased capacity to lay down lean tissue instead of fat reserves. Fattening pigs are growing to 120 kg in around 25 weeks, with a conversion rate of under 3 kg concentrated feed to 1 kg live weight. With regard to behaviour, the social structures are said to be relaxed in the domestic pigs, and aggression, flight distance and motility are lower than in its wild counterpart (Hemmer, 1990). On the other hand, productivity is much higher. Domestic sows have more litters over a given period than wild sows, and in addition their litter numbers 8-12 or even more whereas that of wild sows is 4-6.

Modern husbandry

The intensive management systems that have been developed during the past decades differ in many respects from the former outdoor systems in which pigs were kept in a practically wild state. For example, the common commercial practice is to wean at 3 or 4 weeks of age, although piglets left with their mother are weaned between 14 and 17 weeks of age (Jensen, 1986). Early weaning of piglets is often practised to increase the number of litters which a sow may have over a given period. It induces a number of specific behaviours in piglets such as belly-nosing and sucking of other piglets (Fraser, 1978). During the growth of the piglets to slaughterweight, they may be mixed into new groups several times to create fattening groups that are made up of individuals who are very similar as to breed, size, and age. Mixing unfamiliar pigs will result in fighting to establish a new dominance hierarchy, and they no longer fight once dominance has been settled (Meese and Ewbank, 1973). However, instability has been reported (Meese and Ewbank, 1972) and it has been shown that up to six weeks after mixing elevated levels of agonistic behaviours can be found (Ekkel et al., 1997). Competition over e.g. food may cause aggression in established groups (Fraser et al., 1995).

With respect to the housing methods, fattening pigs are usually kept in pens with fully or partly slatted floors and without substrate available. The minimum required space for a fattening pig weighing between 85 kg and 110 kg is currently 0.65 m^2 in the EC (EC Directive, 1991b) and 0.7 m^2 in the Netherlands (Varkensbesluit, 1994). It has been shown that this type of housing affects the behaviour of pigs compared to extensive housing systems. Schouten (1991) showed that piglets reared in commercial farrowing crates perform less exploration and more manipulation of littermates and the sow than piglets reared in large straw pens. Also during the period from weaning to

slaughter, barren housing conditions result in more harmful social and aggressive behaviour than large straw pens (Beattie et al., 1995; Lyons, 1995; Petersen, 1995). The type of rearing house can also have long-term effects at first farrowing in gilts. Gilts reared in crates show high levels of restlessness before and during farrowing compared with gilts reared in straw pens (Schouten, 1991).

Preslaughter treatment

When a fattening pig has reached slaughter weight, it is transported to the slaughterhouse. The most common means of transport for slaughter pigs is the lorry. As export of live animals within the European Union is allowed, some of the pigs will be transported over long distances. In 1996, 2.7 million pigs were exported from the Netherlands, of which 70% to Germany and 10% as far as Italy (PVE, 1997). Within the Netherlands, the distance between farm and slaughterhouse is limited and transport will generally not last longer than three hours. Upon arrival at the slaughterhouse, pigs are unloaded and moved by slaughterhouse employees into a holding pen in lairage. The main purpose of a lairage is to act as a holding area, where a reservoir of animals can be maintained so that the dressing line can operate at a more or less constant speed irrespective of variations in the delivery of pigs. A second function of lairage is to let the animals recover from transport and in general, about two hours of rest are maintained (Lambooij, pers. comm). After this period, the pigs are moved to the stunning area. Electrical stunning is the principal method used in most European countries. The stunning current is usually applied across the head, leading to a temporary stun. In small slaughterhouses, the method will be carried out manually with the pigs free-standing on the floor of the stunning pen. In large slaughterhouses, pigs are driven one after another in a restraining conveyor in which they will be automatically stunned. Bleeding must be started as soon as posible after stunning (EC directive, 1993).

Assessment of welfare during preslaughter treatment

The EC Directives on the protection of animals during transport (EC Directive, 1991a) and at the time of slaughter (EC Directive, 1993) require national legislation to become effective. Because most countries have not yet implemented the directives in laws and legislation is often based on existing practices, preslaughter treatment is still likely to affect the welfare of pigs. As Duncan and Fraser (1997) pointed out, science cannot provide a purely objective measurement of welfare because the conclusions we

draw about an animal's welfare are based on value judgements as well as knowledge. However, welfare can be assessed by using a large number of variables (Broom and Johnson, 1993). Several measures of poor welfare concern long-term responses, such as a reduced ability to grow or breed, immunosuppression, disease and abnormal behaviour patterns. Welfare indicators for preslaughter treatment will obviously concern short-term responses, since there are usually just a few hours between loading at the farm and the time of slaughter. Frequently used indicators of acute stress are behavioural and physiological responses.

Behavioural responses

Behaviour is one of the most easily observed indicators of welfare: it provides information about the animal's needs, preferences and internal states (Mench and Mason, 1997). Changes in the frequencies of social and non-social behaviours or suppression of behaviours can provide cues about welfare problems. Behaviour observations can show which animals are likely to be most affected by pre-slaughter treatment. Animals which are very disturbed by these conditions are likely to show less normal behaviour such as exploration and either overreact or do not respond to events around them (Fraser and Broom, 1990). Behaviours that are indicative of fear and disturbance are e.g. unwillingness to move, escape and avoidance. Signs that are associated with pain and illness are e.g. trembling, vomiting, panting and difficulties with moving. With regard to transport, observations may be related to the change of position and resting behaviour. Factors such as density, micro-climate and roughness of the journey affect the activities of pigs (Lambooij, 1995). Much attention has been paid to the ease of loading, unloading and moving pigs and environmental factors influencing it. Pigs have difficulties climbing ramps and therefore hydrolic lift pens for loading and unloading are recommended (Grandin, 1983). Races or passageways which are narrow or incorporate sharp bends reduce the ease of movement and thus the time needed to pass through (Warris et al., 1992a). Pigs move more readily from a distinctly darker area into a brightly illuminated area (Van Putten and Elshof, 1978). Grandin (1983) mentioned that shadows will impede the movement of pigs, but Tanida et al. (1996) showed that 1-week old piglets did not respond to shadows and lines across the floor, and that it may be the fence poles themselves rather then their shadows at the floor that stop the animals. Also noise is suggested to frighten pigs in lairage (Grandin, 1983). The behavioural consequences of mixing in lairage have been studied by Moss (1978), who found that intensive fighting occurred in the first half

hour of mixing in groups of 20 pigs and that after one hour of penning the majority of pigs were resting.

Physiological responses

Acute stress is known to affect a large range of physiological variables. A frequently monitored physiological response to acute stress is a change in activity of the autonomic nervous system. The balance between the sympathetic and para-sympathetic branches of the system determines cardiac output. Sympathetic nerve stimulation will increase heart rate, while activity of the vagus nerve slows the heart. An increased sympathetic activity results in increased plasma levels of catecholamines: noradrenaline is principally released by the nerve endings of the sympathetic nervous system whereas the adrenal medulla secretes mostly adrenaline. Activation of the sympatho-adrenal system in response to acute stress is known as the emergency reaction, and functions to mobilise the body's resources for action.

Heart rate measurements have been used to evaluate the effect of loading and transport. The highest peaks in heart rate occurred during loading and unloading (Augustini and Fisher, 1982; Villé et al., 1993). Transport increased heart rate compared to previous resting levels (Stephens and Rader, 1982). With regard to such studies of heart rate, it should be kept in mind that changes as a result of physical activity cannot be distinguished from changes due to emotional responses.

The effect of transport on catecholamines in individual pigs was measured using blood sampling via a jugular catheter (Dalin et al., 1993), showing that transport increased adrenaline levels while noradrenaline levels fluctuated. Troeger (1989) used blood sampling from the ear vein in lairage and showed that forced driving in groups led to higher adrenalin levels than careful driving. It should be noted that the possibilities to obtain blood samples in groups of pigs via catheters are limited because pigs are likely to chew and destroy the catheters of their groupmates. Alternatively, post-slaughter blood samples can be used to assess catecholamines, but both electrical and mechanical stunning methods can cause dramatic increases in catecholamine levels (Shaw and Tume, 1992).

Measurements of activity in the hypothalamic-pituitary-adrenal (HPA) system are also very useful in the assessment of welfare problems due to acute stress. Upon the animal's exposure to a stressful stimulus, corticotropin releasing hormone (CRH) is released from the hypothalamus and stimulates the release of adrenocorticotropin hormone (ACTH) from the anterior pituitary. In turn, ACTH stimulates the release of

glucocorticoids (cortisol and corticosterone) from the adrenal cortex. Glucocorticoids exert a negative feedback on the brain and pituitary in order to regulate the activity of the HPA axis. The axis displays a circadian rhythm in basal activity, with elevated concentrations of ACTH and glucocorticoids in the morning that decrease during the day.

Glucocorticoids function to promote the transformation of non-sugars into sugars and thus provide the body with rapidly mobilised sources of energy. They synergize with the sympathetic nervous system in increasing heart rate and blood pressure, as a means to deliver the mobilized energy substrates to the muscles more rapidly. The HPA axis and the sympatho-adrenal axis influence each other: adrenaline released from the adrenal medulla can evoke ACTH release from the pituitary and glucocorticoid-release from the adrenal cortex, and CRH stimulates the release of adrenaline and noradrenaline.

At moderate to high levels, glucocorticoids generally suppress endocrine, renal, immune and neural defence mechanisms, and it is suggested that elevated levels of corticosteroids in acute response to stress serve to suppress the body's normal defences against stress, preventing those defences from overshooting and themselves threatening homeostasis (Munck et al., 1984).

Peripheral concentrations of glucocorticoids are valuable indicators of stress, because cortisol shows a graduated response, depending on the severity of the stressor. Comparing the cortisol increase of a group of animals subjected to a series of stressors can be a useful means to assess their aversiveness (Terlouw et al., 1997).

Once secreted from the adrenal gland, most of the glucocorticoids bind with high affinity to a binding globulin. The free, i.e. unbound fraction of the glucocorticoids, is the biologically active form. Free cortisol can also diffuse to saliva and its concentration in saliva closely approximates the unbound plasma level (Vining and McGinley, 1984). The rate of equilibrum of cortisol between blood and saliva is less than 5 minutes (Vining et al., 1983). Collection of saliva is an easy, non-invasive procedure and therefore very useful to measure cortisol in the pig (Parrott and Misson, 1989; Parrott et al., 1989). Sampling saliva in pigs to measure cortisol levels has proved to be a practical alternative for sampling blood in evaluating transport (Becker et al., 1985; Nyberg et al., 1988). Cortisol levels in plasma appear to be unaffected by stunning methods and measurement of cortisol in post-slaughter blood samples can be used to assist in evaluation of abattoir treatments (Shaw and Tume, 1992). For example, Warriss et al. (1992b) concluded on the basis of cortisol levels in

post-slaughter blood samples that after two to three hours in lairage basal levels are reached again and therefore a resting period of this duration is required from the welfare point of view.

Individual differences in behavioural and physiological responses

The magnitude of the stress response in a certain condition will also depend on the characteristics of the individual animal. Individual differences in the way animals adapt to environmental challenges are well documented in laboratory animals (Bohus et al., 1987; Benus et al., 1987). Whereas some individuals react in a behaviourally and physiologically active way, others use more passive modes of reaction. These individual characteristics can be related to genetic and ontogenetic factors (Benus et al., 1991), early life experiences (Mendl and Paul, 1991) and recent life experiences (Van Oortmerssen et al., 1985). Also pigs have been shown to express considerable individual variability in response to environmental stimuli. Some authors state that different behavioural and physiological strategies remain constant within individual pigs regardless of context (Lawrence et al., 1991; von Borell and Ladewig, 1992; Mendl et al., 1992; Hessing et al., 1994) while others reject this (Forkman et al., 1995; Jensen et al., 1995). Individual differences in behaviour and physiology may have consequences for the ability of pigs to cope with unfamiliar stimuli such as pre-slaughter treatment.

Preslaughter treatment and meat quality

The behavioural and physiological responses to preslaughter treatment not only are indicators of welfare, but may also have an effect on perimortem muscle metabolism and thereby on meat quality. Before discussing how this effect may be achieved, a brief explanation should be made of the relation between muscle metabolism and meat quality.

Perimortem muscle metabolism and meat quality

In the live animal, carbohydrate metabolism is the main source of energy generation, i.e. ATP production, during muscle exercise (Stryer, 1981). In this process, glycogen is broken down to glucose-1-P units, which are subsequently converted into glucose-6-P units. In the glycolytic pathway glucose-6-P is transformed into pyruvate, and under aerobic conditions further processed via the cytric acid cycle and oxidative phosphorylation, ultimately leading to a high energetic yield of ATP. At

high energy demands during strong exercise, the oxygen supply may become insufficient, and pyruvate will be converted under anaerobic conditions into lactate. Lactate lowers the muscle pH, and enters the blood stream in order to be converted into glucose by the liver.

After slaughter, breakdown of glycogen to lactic acid is the only energy-yielding metabolic pathway available in the anaerobic interior of muscles (Tarrant, 1989). The increase in lactate will lead to a decrease in muscle pH. The breakdown will continue until no more glycogen is available, or until at a pH of ca. 5.4 the activity of the enzymes involved is stopped. The disappearance of ATP will coincide with the onset of rigor mortis, i.e. the rigidity of the musculature.

The processes described above may vary between individuals in the extent of the changes and the rate at which they continue. This variation is responsible for the spectrum of meat quality ranging from PSE (pale, soft, exudative) to DFD (dark, firm, dry). PSE meat is characterized by a rapid rate of pH fall after slaughter, so that the pH reaches a low value while the temperature of the carcass is still high. The meat is pale and watery as a consequence of protein denaturation (Sybesma, 1976). PSE meat has a poor acceptability with consumers and leads to poor processing and cured products (e.g. bacon). DFD pork occurs when muscle glycogen is depleted before slaughter (Tarrant, 1989). Post-mortem acidification is curtailed and the muscle's ultimate pH will be high. DFD is discounted because of its unattractive colour and texture and its shelf-life is reduced considerably (Newton and Gill, 1981).

Preslaughter factors influencing meat quality

The influence of preslaughter treatment on DFD is well understood. Depletion of muscle glycogen by metabolic exhaustion can be induced by e.g. long transport or mixing the animals before slaughter (Tarrant, 1989).

The factors that trigger an extremely rapid glycolysis in the muscle after slaughter and lead to PSE seem to be more complicated and encompass both genetic and environmental factors (Cassens et al., 1975). Genetic factors other than the halothane gene are involved, as some breeds are more prone to develop PSE than others (Monin and Sellier, 1985). In addition, there seem to be many environmental factors that influence the incidence of PSE. For example, the housing systems in which fattening pigs are kept are thought to influence indirectly the responses to preslaughter treatment and subsequent meat quality (Henry, 1993; Barton-Gade and Blaabjerg, 1989). However, the acute stress encountered during short-term transport

and in lairage is believed to be the major cause of PSE meat. Behavioural and physiological responses to acute stress could lead to properties of PSE via an associated increase in muscle exercise, muscle temperature, or elevated hormone levels. The relative effect of ante-mortem muscle exercise and temperature on post-mortem glycolysis and meat quality have been elegantly tested by Klont et al. (1995a, 1995b) in pigs that were anaesthetized 45 minutes before slaughter. Stimulating muscles by electric pulses resulted in a lower water-holding capacity and a lighter meat colour. Anaesthetized pigs that were covered by a blanket in which water of different temperatures was circulated, showed paler meat with increased muscle temperatures. However, in both experiments post-mortem glycolysis was not changed. The authors concluded that muscle pH can already be low at slaughter and thus is not only the result of an increased post-mortem glycolysis. Furthermore, muscle temperature in itself is an important factor in determining post-mortem protein denaturation and thus colour and water-holding capacity.

The hormones released in response to preslaughter treatment may influence meat quality. Sympathetic arousal and adrenaline release can trigger a rapid glycogenolysis and excessive lactate production, thus favoring the development of PSE (Tarrant, 1992). The relationship between cortisol and meat quality parameters is conflicting (Cassens et al., 1975) and there may be an absence of aberrant meat quality in pigs with high cortisol levels (Gregory et al, 1987). However, because the HPA axis and the sympatho-adrenal axis influence each other, increased HPA-axis activity can stimulate adrenaline release. Furthermore, Shaw and Tume (1992) concluded in a review that the absence of a clear relationship between plasma constituent indicators of stress and meat quality indicators of stress is not surprising in view of the transient nature of changes in concentrations of blood constituents.

Thus, preslaughter treatment that leads to an increase in sympatho-adrenal and HPA-axis activity, increased exercise or elevated body temperature, can consequently lead to aberrant meat quality. Several recommendations regarding transport and lairage considering these factors have been made. Pigs should be fasted for 12-24 hours before slaughter, as this is thought to reduce transport death, travel sickness, and the percentage PSE-meat (Eikelenboom et al., 1990). During transport, stocking density should be low enough to allow the pigs to rest (> 0.51 m^2/pigs for live weight > 120 kg, EC working group, 1992), and an environmental temperature of approximately 16 °C with a low air velocity resulted in best meat quality (Lambooij et al., 1987). Warris (1987) concludes in a review on lairage time that a short period in

lairage may reduce the incidence of PSE carcasses, but the length of lairage is ill-defined for different types of pig. Prolonged lairage may lead to problems with DFD-meat, particularly in pigs fasted before transport or fatigued by transport. If pigs are showered with cold water in lairage, this will lower the body temperature and PSE incidence decreases (Smulders et al., 1983). Fighting due to mixing pigs in lairage leads to both more PSE and DFD (Karlsson and Lundström, 1992).

Aim of this thesis

In view of the above the purpose of this thesis is to gain more insight in the relative contribution of common preslaughter treatment factors in evoking behavioural and physiological responses indicative of reduced welfare. Furthermore, attention is paid to the effects of conditions during the fattening period on responses to preslaughter treatment, and to the consequences for meat quality.

During the past decades, slaughterhouses have increased in size and consequently the number of pigs slaughtered became higher. To explore the current situation, field observations were conducted on pigs held in lairage at several slaughterhouses. In Chapter 2, results of this study are described with emphasis on slaughterhouse husbandry practice, the level of agonistic interactions between pigs in the holding pen and individual differences in aggressive behaviour, and resulting skin damage after slaughter.

In Chapter 3, main factors of preslaughter treatment, i.e. transport followed by driving and mixing, were simulated in an experimental setting and the responses of pigs were investigated. Individual differences in aggression during mixing were related to agonistic behaviour shown previously in the home pen and skin damage after mixing. The appendix to Chapter 3 lists the effects of transport followed by driving and mixing on several meat quality parameters.

Porcine meat quality can be predicted to a certain extent by studying properties of the skeletal muscle in live pigs (Lahucky, 1987). Muscle samples are usually collected by a "shot-out" with a specially adapted common slaughter pistol with a cannula on the top (Lahucky et al., 1982; Wegner and Ender, 1990). To estimate whether this 'shot biopsy' method could be used in our project, Chapter 4 describes behavioural and physiological responses of slaughter pigs to the biopsy.

The aim of the study presented in Chapter 5 was to investigate the impact of slaughterhouse sounds on behavioural and physiological responses of pigs. Pigs were exposed to one of the following sounds, recorded in a large slaughterhouse: Pigs

driven to the restrainer and Machines in lairage. As controls, White noise, or No sound were used.

The effects of regular moving and handling during the fattening period on responses of pigs during preslaughter treatment, and the consequences for meat quality are described in Chapters 6. Chapter 7 describes the differences between pigs that were either raised in intensive housing conditions or in less intensive conditions.

In Chapter 8, the major findings of Chapers 2-7 are summarized and discussed.

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Chapter 2

Observations on behaviour and skin damage of slaughter pigs and treatment during lairage

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Abstract

In five Dutch and four Belgian slaughterhouses the following elements were studied: 1) slaughterhouse husbandry practice, 2) agonistic behaviour in groups of pigs, and 3) indices of skin damage following slaughter recorded on a relative scale of 1-4 where 4 indicates severe damage. Variation in slaughterhouse husbandry practice was considerable (stocking density range, 1.0-3.1 pigs/m^2; range of time spent in lairage prior to slaughter, 0-3 h). Brooms and/or electric goads were used to move pigs in all slaughterhouses and in one Dutch abattoir handling with sticks seemed to contribute considerably to skin damage. The level of agonistic behaviour was lowest during the first ten min in lairage. In the Dutch slaughterhouses, large individual differences in aggression between pigs were observed. Pigs in Belgian slaughterhouses were tranquillized prior to transport resulting in generally low levels of agonistic behaviour. The percentage of resting animals increased steadily up to an average of 36% in the Dutch and 45% in the Belgian abattoirs after 1.5 h. In both the Dutch and the Belgian slaughterhouses, skin damage was higher in the front (2.3 $\pm$ 0.1 and 2.1 $\pm$ 0.3 respectively) and in the middle region (2.2 $\pm$ 0.2 and 2.1 $\pm$ 0.2 respectively) than in the hind region (1.7 $\pm$ 0.1 and 1.4 $\pm$ 0.2 respectively) of the pig. For the Dutch slaughterhouses skin damage was significantly associated with time kept in lairage ($P < 0.05$) and stocking density ($P < 0.05$). It is suggested that to decrease aggression and skin damage and thus to increase welfare in the visited Dutch slaughterhouses, stocking density should be lower and pigs should be slaughtered as soon as possible after arrival.

Introduction

The treatment of slaughter pigs during lairage at the abattoir may cause psychological and physical stress, and may also lead to deterioration in meat quality (Mormède and Dantzer, 1987; Tarrant, 1992). Rough, careless handling should be prevented as this is often a source of bruising, particularly at unloading (Tarrant, 1989). A period of rest in lairage is believed to lead to a recovery from the stress of transport and consequently in the production of better meat (Warris, 1987). Two to three hours of rest reduce cortisol levels and are therefore recommended with regard to welfare (Warris et al., 1992). Unfamiliar animals should not be mixed during lairage as they will fight to establish a new social dominance order (Guise and Penny, 1989; Moss, 1978). Fighting gives rise to an increased incidence of skin damage and prevents resting behaviour (Barton-Gade et al., 1992). Apart from ethical considerations and public image, it is in the interests of slaughterhouse management to take the welfare of pigs into

account in order to process carcasses without bruises and therefore profit from resultant meat quality.

In addition to slaughterhouse husbandry practice, individual responses of pigs may play a role with regard to their welfare. In pigs, it is well known that individual differences in behavioural responding to stress exist. Recent studies show different behavioural and physiological strategies which are consistent within individual pigs (Lawrence et al., 1991; Mendl et al., 1992; Hessing et al., 1994). These individual differences may have consequences for the coping capacity of individual pigs in specific stressful situations. For example, when groups of unfamiliar pigs are mixed, the extent to which an individual will engage in agonistic encounters may vary and may result in different physiological changes and/or physical damage (bruises).

Field observations were conducted on pigs held in lairage at various slaughterhouses in order to examine 1) slaughterhouse husbandry practice, 2) the level of agonistic interactions in the holding pen and individual differences in aggressive behaviour, and 3) skin damage after slaughter. Although it was not possible to get reliable information on the origin and already existing skin damage of the animals, an attempt was made to relate skin damage to treatment and aggression.

Materials and methods

Five Dutch (hereafter referred to as A-E) and four Belgian slaughterhouses (hereafter referred to as F-I) were visited in the mild winter of 1993/1994. The number of pigs slaughtered per hour for slaughterhouses A-E was about 600, 750, 350, 350 and 350, respectively; for F-I the number was 250. Visits to three of the Dutch slaughterhouses (A, B and C) were repeated twice.

Subjects

Four groups of pigs were followed during each visit. Around 08.00 h, two groups of pigs were observed simultaneously during lairage (one by recording on videotape, one by live observation) and skin damage was scored post-mortem after scalding and evisceration. Subsequently, around 12.00 h two other groups were followed in the same way. No information was available concerning the type and number of pens the observed pigs were kept in during fattening, their treatment during loading, or already existing skin damage on arrival at the slaughterhouses.

Slaughterhouse husbandry practice

The kind of tools used when moving pigs (e.g. electric goads and sticks) was recorded. Lairage time (time kept in the holding pens), number of pigs per group and density of pigs/m^2 were determined.

Agonistic behaviour

During each slaughterhouse visit, behaviour of two out of four groups in lairage was recorded on video tape after the pigs were placed in the holding pens, one around 08.00 h and one at around 12.00 h. On arrival at the slaughterhouse, 30 individuals in each video-recorded group were given a colour code on the back with a standard marker spray, for ease of identification. A GR-M3 camcorder was mounted above the pen providing a view area which encompassed 90% of the pen area. For each pen, recordings were collected for 1.5 h in real-time. Tapes were subsequently viewed and analysed with The Observer / Video Tape Analysis System (Noldus, 1991). Frequency and duration of all agonistic interactions were recorded, and if a marked individual was involved, its code as well as its role (aggressor or receiver) was recorded.

In addition to the video-observed groups, two other groups were observed directly during each slaughterhouse visit for five bouts of 10 min each. The first bout started when the pigs entered the holding pen, the second started at t = 20 min, the third at t = 40 min, the fourth at t = 60 min, and the fifth at t = 80 min. All agonistic interactions were recorded with The Observer 2.0 on a Psion Organiser LZ 64 hand-held computer. Duration of the interactions was calculated. At the beginning and end of each 10-min period, the number of animals resting (sitting and lying) was also recorded.

Skin damage

After scalding and evisceration, skin damage in front (cranial to the caudal point of the shoulder), middle and hind region (caudal to the hipbone) was assessed subjectively employing a 4-point scale which takes product utilization into account (Barton-Gade, 1993): 1, no skin damage; 2, slight skin damage; 3, skin damage affecting quality; 4, extreme skin damage with possible rejection of tissue.

In Dutch but not in Belgian slaughterhouses, pigs generally had eartags so that marked animals from the groups recorded on video could be individually recognized after slaughter.

Statistical analysis

Variables related to skin damage were initially analysed with a mixed analysis of variance model (Searle et al., 1992, Ch. 1). Group means were analysed. Slaughterhouse effects were entered as fixed effects in the model. These effects encompass all effects particular to slaughterhouses, including e.g. effects of employees and loading facilities. Other fixed effects include e.g. lairage time, which was entered as a co-variate. Visits within slaughterhouses were entered as random effects. Group sizes were entered as weights for the residual error terms in the model. So:

$$y_{ijk} = \mu + s_i + ß\, x_{ijk} + v_{ij} + e_{ijk},$$

where y_{ijk} is the mean for the k-th group in the j-th visit to the i-th slaughterhouse based on on n_{ijk} observations, μ is an overall mean, s_i is the effect of the i-th slaughterhouse, x_{ijk} is the lairage time with coefficient ß, v_{ij} is the random effect for the j-th visit, and e_{ijk} is the residual error term.

Terms v_{ij} and e_{ijk} are assumed to be mutually independent, with means 0 and variances σ^2_v and σ^2_e/n_{ijk}, respectively. Means relating to the same visit, based on n and n´ observations respectively, have a common random effect for that visit and are positively correlated with correlation $\rho = \sigma^2_v / \sqrt{\{(\sigma^2_v + \sigma^2_e/n)(\sigma^2_v + \sigma^2_e/n')\}}$. Components of variance were estimated with restricted maximum likelihood (REML) (Pattersen and Thompson, 1971; Searle et al., 1992, Ch. 6). Calculations were performed with REML facilities of Genstat 5 (1993). The intra-visit correlations ρ proved to be very small. Therefore, subsequent analyses were performed without the random effects for visits in the model. This reduces the analysis to an unbalanced weighted analysis of variance. Various models were fitted to the data employing regression routines of Genstat 5, with appropriate dummy variables for the factors in the models. As a descriptive addition to these analyses, Spearman correlations were calculated between variables using group means. Furthermore, estimated means for duration of agonistic interactions during lairage time were calculated over groups and slaughterhouses with a mixed model analysis.

In addition to the analysis of group means, Spearman correlations between variables (e.g. skin damage and aggression received) were calculated per group recorded on video. These correlations were averaged (e.g. over groups within slaughterhouses). Seven out of 22 video recordings in Dutch lairage had to be omitted from analysis due to poor tape quality (one out of six for each of the slaughterhouses A, B, and C, and two for each of slaughterhouse D and E). The average correlation, based on say m groups of sizes $n_1 \ldots n_m$, was referred to a normal distribution with mean 0 and variance of $\Sigma(n_k\text{-}1)^{-1}/m^2$, to

test whether the variables were significantly related. The variance under the null-hypothesis of no relationship refers to the situation that rank numbers of one variable are randomly combined with rank numbers of the other variable concerned (Conover, 1980).

Results

Dutch slaughterhouses (A - E)

Slaughterhouse husbandry practice. Almost all transport-workers used electric goads to unload pigs from lorries. Employees of the slaughterhouses used electric goads and brooms to move the pigs. In slaughterhouse D, rubber sticks were used. Only in slaughterhouse C driving was done quietly without using tools. Speed of driving did not seem to differ from that in other slaughterhouses. In all slaughterhouses, electric goads close to the restrainer were powered by electric mains, showing that management allows their usage.

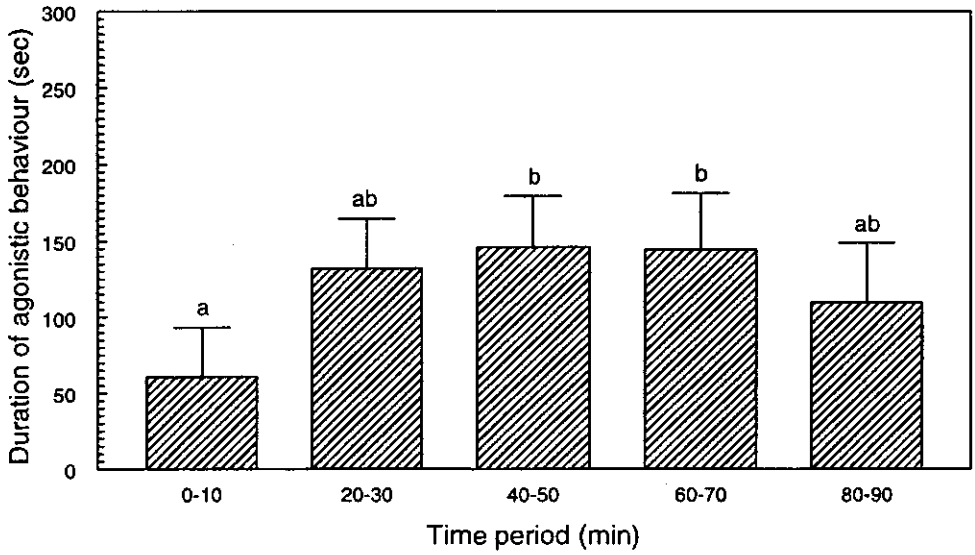

Fig. 1. Mean (± SEM) total duration of agonistic interactions in 10-min bouts during lairage time for an average group in Dutch slaughterhouses. Means lacking a common superscript differ significantly ($P < 0.05$).

Only batches of pigs from the same original producer were mixed in a pen. The number of pigs per producer varied considerably. As a consequence, mean group size in a holding pen was 50 ± 2 pigs, with a minimum group size of 27 and a maximum group size of 90, and density in the holding pens varied between 1.0 and 2.7 pig per m^2.

Lairage time varied. In the small slaughterhouses, supply of pigs was sometimes insufficient for a number of hours, causing the number of pigs in lairage to be depleted. New pigs arriving at the slaughterhouse were then driven immediately to the stunning point, as a steady stream of animals delivered to the stunning point was required. If the supply of pigs was sufficient, pigs would stay in lairage for up to three hours. Mean lairage time in slaughterhouses A, B and C was almost two hours, mean lairage time in slaughterhouses D and E was about one hour.

Behaviour. The total duration of agonistic interactions at 40-50 min and 60-70 min was significantly higher than during the first 10 min after introduction in the holding pen (Fig. 1). The occurrence of aggressive behaviour was by no means evenly distributed amongst individuals (Fig. 2a and 2b). In each group, about five pigs were the major aggressors. Their aggression was always directed to more than one animal. The pigs which received the majority of the aggression were always attacked by more than one animal; 41.3 ± 8.3% of the animals in a group were not involved in agonistic interactions at all. Pooled Spearman correlations were calculated between aggression performed by a pig and aggression received by that individual (Table 1) to form an impression of the relationship within groups. For all three slaughterhouses A, B, and C these correlations were significant.

Table 1. Spearman rank correlation coefficients (r_s) for 3 Dutch slaughterhouses between (1) total frequency of aggression performed by a pig and total frequency of aggression it received, and between (2) total duration of aggression performed by a pig and total duration of aggression it received. Correlation coefficients were calculated for 5 groups within each slaughterhouse and pooled

Abattoir	Frequency performed aggression - frequency received aggression	Duration performed aggression - duration received aggression
A	0.44^{***}	0.53^{***}
B	0.61^{***}	0.64^{***}
C	0.57^{***}	0.59^{***}

*** Significant at $P < 0.001$.

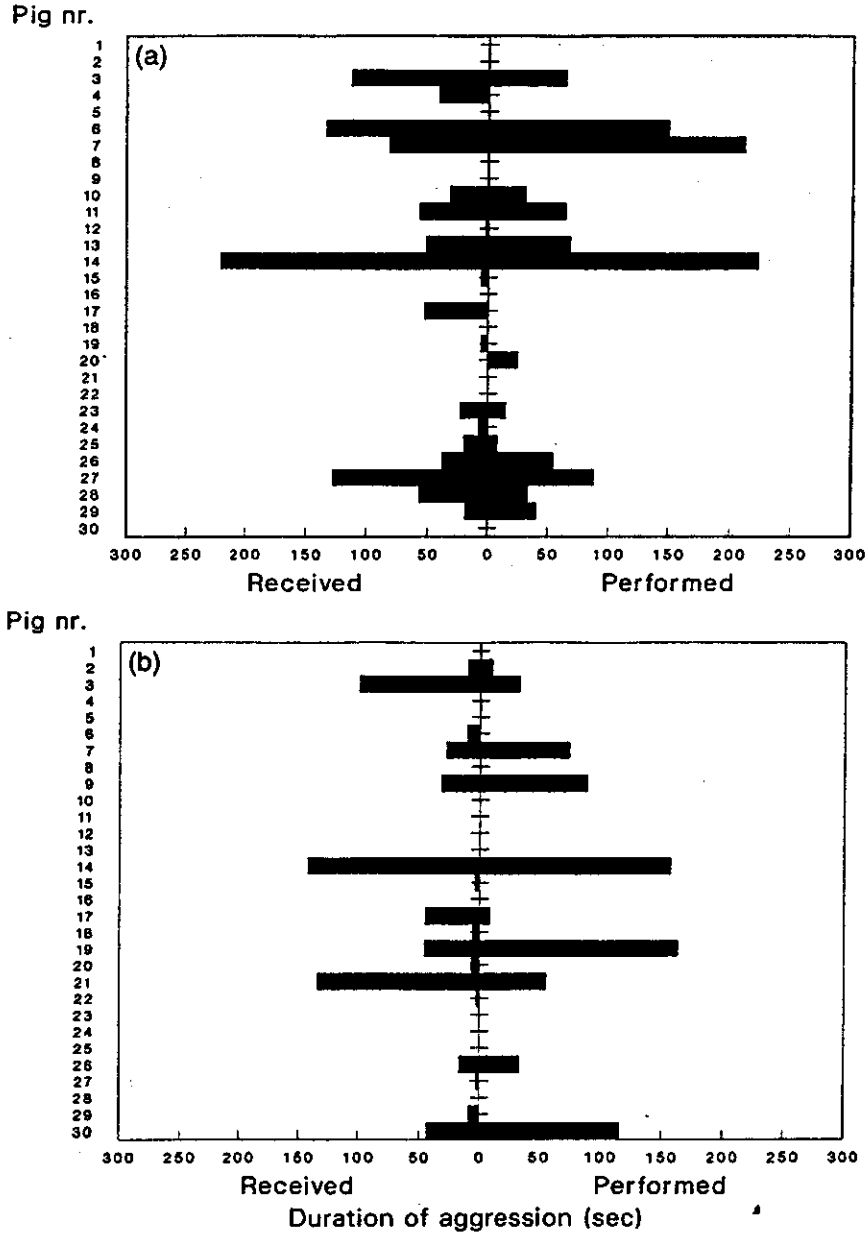

Fig. 2. Total duration of aggression performed and received by each marked animal in two individual groups (n = 30) recorded on video during lairage time (60 and 90 min, resp.) in Dutch slaughterhouses.

Immediately after the pigs were introduced in the holding pen, they started to lie down along the fences. The number of pigs lying down gradually increased during lairage time (Fig. 3). The highest percentage of pigs observed resting was 87%; the mean percentage after 1 h was 27%, and after 1.5 h this had increased to 36%.

Skin damage. Analysis showed that effects of visits within slaughterhouses were negligible for variables related to skin damage, i.e. components of variance for differences between visits were small compared to the residual components representing variation within visits. Formal F-tests showed that none of the components differed significantly from zero. Therefore, effects for visits were excluded from the models subsequently fitted to the data. This means that subsequent analyses were performed with (unbalanced) analysis of variance models. Skin damage in the hind region was always lower than skin damage in the front and middle region. Mean skin damage score for the front region was 2.3 ± 0.1, for the middle region 2.2 ± 0.2, and for the hind region 1.7 ± 0.1 (Table 2). F-tests for differences between slaughterhouses were significant for all skin damage variables ($P < 0.01$ in all three cases). Pair-wise comparisons showed that slaughterhouse D differed from A, B, and E in skin damage in middle and hind region (Table 2). The scores for D were significantly ($P < 0.05$) higher. In slaughterhouse D rubber sticks were used to drive the animals and with scoring of skin damage, marks left by these sticks in the middle and hind region were clearly visible.

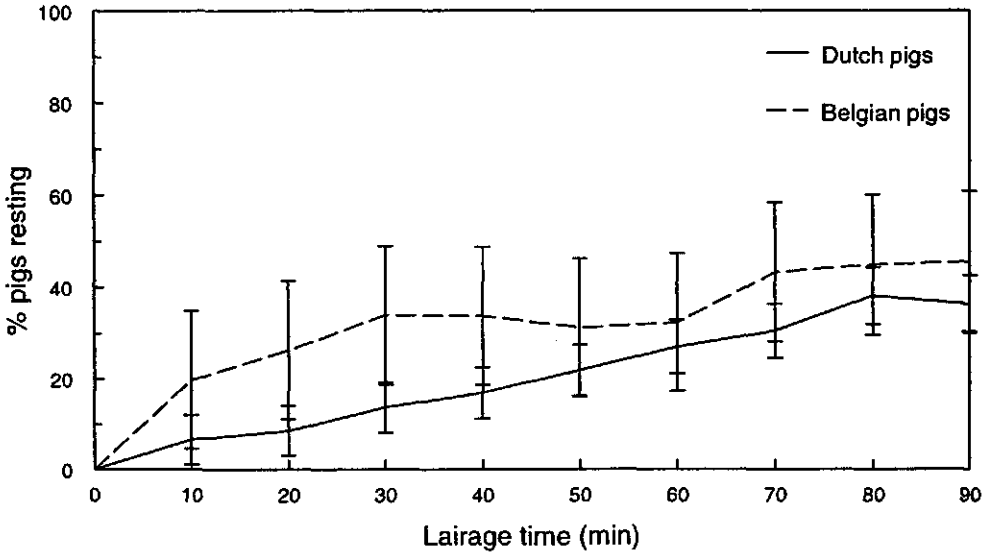

Fig. 3. Mean (± SEM) percentage of animals resting (sitting and lying) during lairage time in Dutch and Belgian slaughterhouses.

Table 2. Skin damage means (±SEM), weighed for group size, for 5 Dutch slaughterhouses.

Abattoir	n	Skin damage		
		Front region	Middle region	Hind region
A	11	$2.3\pm0.1^{a,b}$	1.8 ± 0.1^{a}	1.5 ± 0.1^{a}
B	12	2.1 ± 0.1^{a}	1.8 ± 0.1^{a}	1.6 ± 0.1^{a}
C	12	2.5 ± 0.1^{b}	2.5 ± 0.1^{b}	$1.7\pm0.1^{a,b}$
D	4	2.6 ± 0.2^{b}	2.7 ± 0.2^{b}	2.0 ± 0.2^{b}
E	4	$2.0\pm0.2^{a,b}$	$2.2\pm0.2^{a,b}$	1.5 ± 0.2^{a}
All[1]	5	2.3±0.1	2.2±0.2	1.7±0.1

[a,b] Means within a column lacking a common superscript differ significantly ($P < 0.05$)
[1] Averaging over the means of A...E.

Lairage time was included as a covariable in the model. The corresponding coefficient was significantly different from zero for skin damage in the front region ($P < 0.05$), but not for the middle and hind regions. Thus, lairage time was significantly associated with skin damage in the front region. For the front region, duration of lairage time "explained" only 4.1% of the variance, while addition of the factor for slaughterhouses increased this percentage to 31.4%. Percentages explained for the middle and hind regions were negligible without slaughterhouses in the model, and equalled 46.3 and 23.1% respectively with slaughterhouses included. This shows that lairage time explains only a small part of the variation in skin damage between slaughterhouses. With stocking density as a covariable in the model, the corresponding coefficient was significantly different from zero for skin damage in the hind region ($P < 0.05$), showing that density was significantly associated with skin damage in the hind region. Density explained 16.2% of the variance, while addition of the factor for slaughterhouses increased this percentage to 30.8%. Percentages explained for the front region were negligible and for the middle region 14.6% without slaughterhouses in the model, and 25.5 and 47.1% respectively with slaughterhouses included. In addition to these analyses, Spearman correlations were calculated over slaughterhouses for group means of lairage time, skin damage and density. Correlation between lairage time and skin damage, and between stocking density and skin damage was significant. Density and lairage time were also correlated ($r_s = 0.30$; d.f. $= 40$; $P < 0.05$), and a partial correlation was

determined to form an impression of the relationship, after correction for stocking density, between lairage time and skin damage, and after correction for lairage time, between density and skin damage. Lairage time correlated significantly with skin damage in all parts of the body, whilst density correlated significantly with skin damage in the middle and hind regions (Table 3).

Table 3. Spearman rank correlation coefficients (r_s) between (1) lairage time and skin damage and (2) density and skin damage in 5 Dutch slaughterhouses (d.f. =40). Correlations are based on group means

Variable	Skin damage		
	Front region	Middle region	Hind region
Lairage time	0.39**	0.41**	0.38**
Density	0.05	0.43**	0.39**

** Significant at $P < 0.01$.

Analyses reported sofar were performed on group means. Spearman correlations, pooled per slaughterhouse A, B, and C, as well as pooled over those slaughterhouses, were calculated between total duration of received aggression and skin damage to form an impression of their relationship within groups. Skin damage in the front region correlated significantly with total duration of received aggression for slaughterhouse B, and for slaughterhouses A, B, and C together (Table 4 and Fig. 4).

Table 4. Spearman rank correlation coefficients (r_s) between duration of received aggression and skin damage in 3 Dutch slaughterhouses. Correlations are calculated for 5 groups within each slaughterhouse and averaged per slaughterhouse and averaged over slaughterhouses

Variable	Abattoir	Skin damage		
		Front region	Middle region	Hind region
Received aggression	A	0.15	0.10	0.05
Received aggression	B	0.35**	0.12	0.09
Received aggression	C	0.16	0.01	-0.01
Received aggression	All	0.22**	0.08	0.04

** Significant at $P<0.01$.

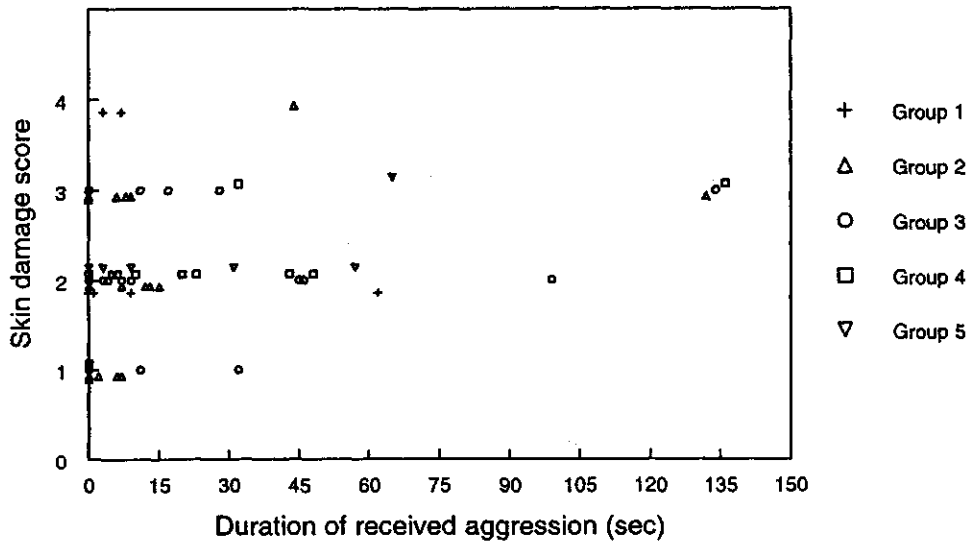

Fig. 4. Skin damage score for the front region in relation to total duration of received aggression in five groups in slaughterhouse B. Pooled correlation was moderate but significant.

Belgian slaughterhouses (F - I)

Slaughterhouse husbandry practice. Most transport-workers used electric goads to unload pigs from lorries. Electric goads and brooms were used to move pigs in lairage. Mean group size in a holding pen was 43 ± 5 pigs, with a minimum group size of 26 and a maximum group size of 90 pigs. Stocking density varied from 1.2 to 3.1 pigs/m^2. Lairage time varied with supply. Mean lairage time was about 1.5 h.

Behaviour. Few agonistic encounters were observed during lairage. Most pigs were crossbreds with Belgian Landrace and Pietrain, known to be relatively vulnerable to transport stress. Transport-workers confirmed that all groups of pigs (except two groups in slaughterhouse G, one directly-observed and one video-observed group) were injected before transport with azaperone, a tranquillizer. As sedation with azaperone lasts for about six h and transport generally lasted about 1 h, pigs were still drugged during lairage and showed low levels of aggression compared to non-sedated pigs (Fig. 5). In general, aggression was lowest in the first 10 min after introduction in the holding pen. In the video-recorded group in slaughterhouse G that was not sedated, aggression performed by individuals correlated positively with aggression received (frequency: r_s = 0.71; d.f. = 28; $P < 0.001$; duration: r_s = 0.64; d.f. = 28; $P < 0.001$).

Pigs quietly lay or stood during lairage time. Mean percentage of pigs resting (sitting or lying) in tranquillized groups was 32% after 1 h and 45% after 1.5 h, slightly higher than mean percentages in the Dutch slaughterhouses (Fig. 3).

Skin damage. F-tests for differences between slaughterhouses were not significant for any of the skin damage variables. Mean skin damage score for the front region was 2.1 ± 0.3, for the middle region 2.1 ± 0.2 and for the hind region 1.4 ± 0.2. Skin damage in the hind region was significantly lower in the Belgian slaughterhouses than in the Dutch slaughterhouses ($P < 0.01$).

Lairage time was included as a covariable in the model. The corresponding coefficient was not significantly different from zero for skin damage in front, middle and hind region, so there was no significant association between lairage time and skin damage. Without slaughterhouses in the model, percentages explained were 3.9% for the front region and negligible for the middle and hind region, and 37.4%, negligible and 32.1% respectively with slaughterhouses included. With stocking density as a covariable, coefficients were not significantly different from zero either. Percentages for all regions were negligible without slaughterhouses in the model, and were 37.2%, negligible and 2.4% with slaughterhouses included. Density and lairage time did not correlate with skin damage to any degree.

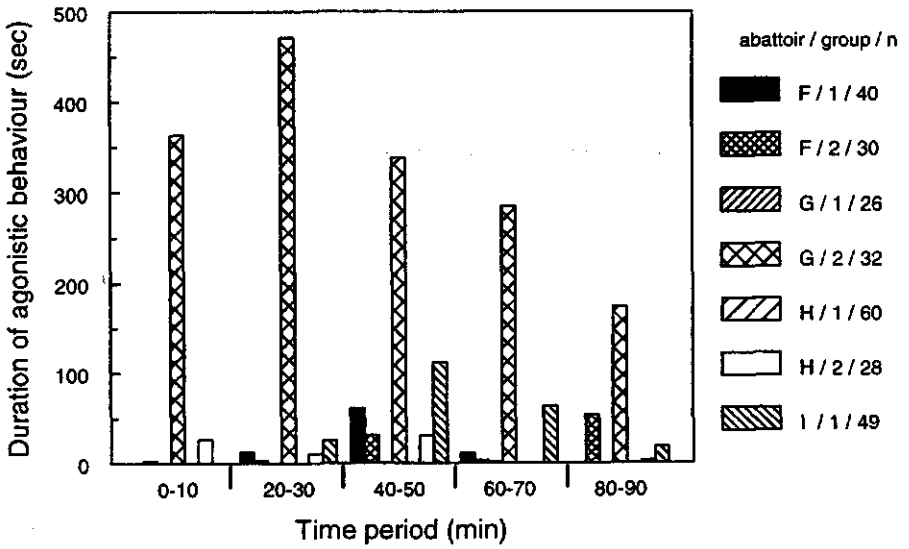

Fig. 5. Total duration of agonistic interactions in individual groups in 10-min bouts during lairage time in four Belgian slaughterhouses. Group 2 in slaughterhouse G was the only group not tranquillized. Group 1 in slaughterhouse F was slaughtered after the fourth period.

Discussion

Repeatedly shocking a pig with an electric goad will increase its heart rate with each successive shock which can be detrimental (van Putten and Elshof, 1978). Using an electric goad may also increase blood spots in the meat (Calkins et al., 1980). However, in this survey almost all transport workers and slaughterhouse employees used electric goads to move pigs. In one slaughterhouse, sticks were used which seemed to contribute considerably to skin damage in the hind region.

It is still common practice to mix different rearing groups of a producer during transport and lairage. Since pigs in general do not fight during transport (Lambooij, 1988), establishing a social dominance order will take place mainly during lairage. In this study, the level of agonistic behaviours was significantly higher at 40-50 min and 60-70 min than during the first 10 min after introduction in the holding pen. This contradicts earlier studies which showed that the majority of aggressive encounters after mixing occur in the first 30 min (Moss, 1978). However, Moss mixed two lots of five pigs, while in the current study groups consisted of at least 26 pigs and consequently more than two rearing lots were mixed. A larger group size with more unfamiliar pigs may well prolong occurrence of fighting. This study showed large individual differences in involvement in agonistic encounters. The association between performed and received aggression suggests that pigs are rarely involved in fights as aggressor or receiver only. The effect of the tranquillizer used on pigs transported to the Belgian slaughterhouses seems mainly to be a reduction of agonistic encounters but without a sizeable increase in the number of resting animals.

Skin damage was higher in the front and middle regions than in the hind region, indicating agonistic encounters as one of the main causes: normally during fighting, bites are targeted mainly at the ears, face and neck (McGlone, 1985). Skin damage in the front region correlated significantly with total duration of received aggression for slaughterhouse B, and for slaughterhouse A, B and C together. Several other studies showed that mixing increased the frequency of skin blemish values (Guise and Penny, 1989; Karlsson and Lundström, 1992). This study supports these results and in addition suggests that skin damage will increase when lairage time increases, as there was a significant association between lairage time and skin damage in the front region in the Dutch slaughterhouses. There was no association between lairage time and skin damage for Belgian slaughterhouses, probably due to little aggression during lairage. The association between density and skin damage in the hind region may result from the

possibility that an increased density will hamper a pig fleeing after being attacked by an aggressor, resulting in bites in the hind region.

A period of rest in lairage is generally recommended to allow the pigs to recover from transport and the associated handling. It is believed that this will lead to the production of better meat (Tarrant, 1989) as well as being desirable from a welfare point of view (Warris et al., 1992). However, present results suggest that, from the point of view of animal welfare, it may be better to slaughter the animals immediately upon arrival.

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Chapter 3

Effects of simulated lairage conditions on the physiology and behaviour of pigs

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Abstract

The response of pigs to two lairage events, driving and mixing, was investigated. Five groups of six and five groups of seven 70-kg pigs were transported for 40 min on a lorry and subjected to one of the following treatments: two groups were driven down a passage (simulating driving in lairage); four groups were mixed for one hour (A and B together, C and D together); four groups received the driving treatment immediately followed by the mixing treatment ('combined treatment'). Behaviour was recorded, skin damage was scored and saliva samples taken for analysis of cortisol. Initial transportation led to increased concentrations of cortisol. Behaviour during driving was not correlated with concentrations of cortisol after driving and cortisol did not increase relative to post-transport levels. Frequency and duration of fighting during mixing were positively correlated with aggression in the home pen and increase in concentrations of cortisol during mixing. One hour after the start of mixing, concentrations of cortisol had decreased relative to post-transport levels. After the combined treatment, all correlations described for the mixed treatment were absent and concentrations of cortisol increased relative to post-transport levels. Skin damage was highest after the mixing treatment. The responses observed indicate that the combined events of driving and mixing, which are very common in lairage, lead to a greater response than in the case of each individual treatment.

Introduction

Slaughter pigs are frequently kept in restricted environments generally with low levels of stimulation. As a result they may have little capacity to adapt to novel stimuli or new environments (Broom and Johnson, 1993). For example, several authors showed that pigs raised in a barren environment, react more strongly towards a novel object (Stolba and Wood-Gush, 1980; Pearce and Patterson, 1993). Pigs may also be more aroused by novel stimuli such as those commonly encountered before slaughter e.g. loading, unloading, transport, driving by unfamiliar stockmen at the abattoir, and mixing with other unfamiliar pigs (Kilgour and Dalton, 1984). These factors are known to have potential effects on behavioural and physiological responses of pigs in lairage (Grandin, 1983). Troeger (1989) has shown that forced driving can result in a significant increase in plasma adrenaline levels when compared with more careful driving. It has also been shown that mixing groups of previously unfamiliar pigs during transport and in lairage may result in fighting (Moss, 1978; Guise and Penny, 1989; Bradshaw et al., 1996a), which can lead to increased skin damage (Karlsson and Lundström, 1992). Recent

research has shown that skin damage increases proportionally to the time pigs spend in lairage (Geverink et al., 1996). Transportation by road has also received considerable attention and is known to be aversive (e.g. Stephens and Perry, 1990; Lambooy and van Putten, 1993; Warriss and Brown, 1994; Bradshaw et al., 1996a, b).

Pigs have been shown to express considerable individual variability in response to environmental stimuli. For example, large individual differences in aggression when pigs were mixed in lairage have been observed (Geverink et al., 1996). Some authors state that different behavioural and physiological strategies remain constant within individual pigs regardless of context (Lawrence et al., 1991; von Borell and Ladewig, 1992; Mendl et al., 1992; Hessing et al., 1994) while others reject this (Forkman et al., 1995; Jensen et al., 1995). However, it is clear that individual differences in behaviour and physiology may have consequences for the ability of pigs to cope with unfamiliar stimuli.

In this study we examined (1) the effects of driving and mixing (following an initial period of transportation) on behaviour and cortisol responses; (2) different individual behavioural and cortisol responses to these treatments and whether these responses were related to pre-treatment behaviours and concentrations of cortisol.

Material and methods

Animals and housing

The experiments were conducted at the University of Cambridge Pig Unit. A total of 65 70-kg pigs (Landrace X Large White) were used in two similar experiments ("replicates"; the time interval between the replicates was 10 weeks). Pigs were weaned and mixed at an age of 4 weeks and housed in groups of approximately 20 pigs. At 15 weeks old subjects were selected at random from 2 weaner groups (without mixing pigs from different groups) and housed in five groups of 2 boars and 4 gilts in the first replicate and five groups of 3 boars and 4 gilts in the second replicate. Each pen had a straw area (3.20 x 2.47 m) and a dunging area (1.23 x 2.47 m). Pigs had free access to water from a nipple drinker. Pigs were given food from a trough twice a day at 08.00 h and 14.30 h (Dalgety Ultrabreed 16 nuts) and the quantity offered was 2 kg per pig per feed.

Behaviour observation in home pen

Each pig was sprayed with a standard colour stockmarker to allow identification during behavioural observation. Behavioural observations were carried out for six days at 16 weeks of age and were always performed by the same observer. Each group was

observed once each day, during a specified 30 min period, with each group being observed during all of the following time intervals over the course of six days: 08.00 - 8.30; 08.30 - 09.00; 13.30 - 14.00; 14.00 - 14.30; 14.30 - 15.00; 15.00 - 15.30. During these periods, continual data were collected for all animals in the observed group. In addition one day before the treatment at 20 weeks, each group was observed from 14.00 to 15.00. The following data were recorded with The Observer 3.0 (Noldus, 1991): frequency of agonistic behaviours (knock, bite, threat, chase, avoid, displace) and activity, i.e. duration of walking or standing expressed as a percentage of total time recorded. Social status of each individual within each group was determined according to the social rank index, as described by Lee et al. (1982).

Salivary cortisol in the home pen

The use of saliva rather than blood for sampling cortisol has been validated in pigs (Parrott and Misson, 1989; Parrott et al., 1989). Saliva samples were collected by allowing the pig to chew on two cotton buds until they were thoroughly moistened (about 1 min). These cotton buds were stored in test tubes, kept on ice and subsequently centrifuged at 5000 g for 5 min to remove the saliva which was stored at -20°C. Concentrations of cortisol were measured using an enzyme-linked immunosorbent assay (ELISA, Cooper et al., 1989). Samples were collected at 8.45, 10.00 and 14.00, the latter immediately prior to feeding, at 17 weeks of age for three consecutive days. The samples at 8.45 and 10.00 matched sampling times at week 20 when the samples were taken before transport and after transport, respectively (see Table 1). In two groups (one in each replicate), a fourth sample was taken at 10.30 which matched the sampling time at week 20 when the 10.30 sample was taken after the driving treatment; in four other groups (two in each replicate) a fourth sample was taken at 11.30 which matched sampling time at week 20 after the mixing treatment; in the four remaining groups (two in each replicate) a fourth sample was taken at 12.00 which matched sampling time at week 20 after the driving and mixing treatment.

Transportation before treatment

When pigs were 20 weeks of age, each group was exposed to 40 min of road transport using a commercial livestock lorry (four-wheeler rigid chassis). A total of 54 km was travelled per replicate. Food was withdrawn on the morning of transport. At 9.00, pigs were loaded onto the lorry. Groups were penned separately (0.60 m^2/pig). Straw was not available on the lorry but sawdust was liberally provided to absorb

moisture. Saliva was collected before and after transport for analysis of cortisol (see Table 1).

Table 1. Time schedule for sampling saliva in the home pen at 17 weeks of age, and during the treatments at 20 weeks of age.

Time	Treatment					
	Driving (2 groups)		Mixing (4 groups)		Driving + Mixing (4 groups)	
	17 weeks+	20 weeks	17 weeks	20 weeks	17 weeks	20 weeks
08.45	saliva	saliva	saliva	saliva	saliva	saliva
- 09.00						
09.15		*transport*		*transport*		*transport*
09.30						
09.45						
- 10.00	saliva	saliva	saliva	saliva	saliva	saliva
10.15		*driving*				*driving*
10.30	saliva	saliva		*mixing*		
10.45						
- 11.00						*mixing*
11.15						
11.30			saliva	saliva		
11.15						
- 12.00					saliva	saliva
|						
- 14.00	saliva		saliva		saliva	

+ Age of pigs

Following this period of transportation, pigs were subjected to one of three treatments: (a) driving (one group in each replicate); (b) mixing (two groups in each replicate); (c) a combined driving and mixing (two groups in each replicate).

(a) Driving treatment. Pigs were vigorously driven twice down a passage 37 m in length (mean duration about 2 min). The stockperson was unfamiliar to the pigs and used a stockboard. The procedure was recorded with four video cameras all arranged at different angles to the passage. From the videotapes, the order of animals in the group was scored every 4 m, along with the number of times a pig was pushed by the stockperson. Saliva samples were taken from each pig after completion of driving (see Table 1).

(b) Mixing treatment. Two groups were separately unloaded after transport and driven a short distance to the mixing pen (5.05 x 2.23 m; 0.94 m^2/pig in first replicate and 0.80 m^2/pig in the second replicate). Behaviour was videotaped for one hour, providing information on agonistic interactions. Tapes were subsequently viewed and analysed with The Observer / Video Tape Analysis System (Noldus, 1991). Saliva samples were taken from each pig immediately after mixing (see Table 1).

(c) Combined driving and mixing treatment. Two groups were separately unloaded after transport and driven through the passage as described in (a) above. Pigs were then driven to the mixing pen. Behaviour was videotaped for one hour, providing information about agonistic interactions. Saliva samples were taken immediately after this mixing period (see Table 1).

Skin damage

Skin damage in the front (cranial to the caudal point of the shoulder), middle and hind region (caudal to the hipbone) was subjectively assessed before and after each treatment on a 4-point scale (Barton-Gade et al., 1996) with 1 = no skin damage and 4 = extreme skin damage.

Statistical analysis

The results from the two replicates were run together and a Wilcoxon's signed-rank test (Conover 1980) was used to analyse differences between cortisol measurements before and after transport (n=65), driving (n=13), mixing (n=26), and the combined treatment (n=26).

Secondly, a Spearman's rank correlation coeffient (Conover, 1980) was employed to describe the relationship between behavioural parameters and concentrations of cortisol. A pooled correlation coefficient was obtained by averaging over separate Spearman coefficients calculated within groups. To test whether a pooled correlation differed from 0, a normal approximation was used, with an approximate variance of $\Sigma(n_i-1)^{-1}m^{-2}$, where n denotes group size and m is the number of groups. The variance under the null-hypothesis of no relationship refers to the situation that rank numbers of one variable are randomly combined with rank numbers of the other variable (Conover, 1980).

Finally, skin damage data were analysed with an analysis of variance model (ANOVA), with a main factor for treatment and a random factor for experimental groups. All calculations were performed with the statistical programming language GENSTAT 5 (1993).

Results

Transportation

Transport resulted in a significant increase in concentration of cortisol (mean level before: 6.30 ± 0.91 nmol/l; after: 18.75 ± 1.87 nmol/l; $P < 0.001$). Cortisol concentrations before transport did not correlate with concentrations after transport (r_s = -0.16, ns).

Treatments

(a) Driving. Following driving, concentration of cortisol did not significantly change relative to concentrations after transport, but levels were still significantly higher than before transport ($P < 0.05$; see Figure 1a). Change in concentration of cortisol was not significantly correlated with cortisol change following transport (r_s = -0.20, ns), mean order during driving (r_s = 0.25, ns), or the frequency of being pushed by the driver (r_s = 0.10, ns). In addition, order during driving was not significantly correlated with social status in the home pen (r_s = 0.25, ns), activity in the home pen (r_s = 0.09, ns) or change in cortisol following transport (r_s = -0.10, ns).

(b) Mixing. Following mixing, the mean concentration of cortisol significantly decreased relative to post-transport levels ($P < 0.05$), but was still significantly higher than before transport ($P < 0.001$; see Figure 1b). Most fights occurred in the first half hour of mixing (total duration of fights for all pigs in first half hour: 1254 ± 16 sec and

total duration of fights in the second half hour 147 ± 82 sec). The frequency and duration of fighting during mixing were positively correlated with the frequency of aggressive behaviours in the home pen (r_s = 0.63, P < 0.01; r_s = 0.44, P < 0.05). The duration of fighting was significantly negatively correlated with decrease in cortisol (r_s = -0.42, P < 0.05), and positively with increase in skin damage (r_s = 0.83, P < 0.01). The decrease in concentration of cortisol during mixing correlated positively with the increase in cortisol during transport (r_s = 0.68, P < 0.01).

(c) Combined driving and mixing. Following the combined treatment, concentration of cortisol increased significantly relative to post-transport levels (P < 0.001; see Figure 1c). Most fights occurred in the first half hour after mixing (total duration of fights in first half hour: 1020 ± 371 sec and total duration of fights in the second half hour 83 ± 59 sec). The frequency and duration of fighting during mixing was not correlated significantly with the frequency of performance of agonistic behaviours in the home pen (r_s = 0.21, r_s = 0.14, ns). The duration of fighting during mixing was not correlated significantly with the increase in cortisol levels (r_s = 0.25, ns) or skin damage (r_s = 0.13, ns). The increase in cortisol levels during the combined treatment was not correlated significantly with the increase during transport (r_s = 0.06, ns).

Skin damage

Mean increase in skin damage in the front region following the mixing treatment (increase with 1.6 ± 0.2 points) and the combined treatment (1.1 ± 0.2) was higher than increase in skin damage after the driving treatment (0.2 ± 0.2; P < 0.05). In addition, the increase following the mixing treatment was higher compared with the combined treatment (0.05 < P < 0.10).

Discussion

The present study suggests that fighting during mixing is predicted by aggressive behaviour shown in the home pen, but this relation is diminished when an additional factor, driving, is added. Cortisol response after combined driving and mixing is higher than cortisol response after the separate treatments.

Transport on the lorry increased the mean concentration of salivary cortisol. This is in agreement with results from other studies for plasma cortisol (Spencer et al., 1984; Nyberg et al., 1988) and salivary cortisol (Zanella and Unshelm, 1994; Bradshaw et al., 1996a; Bradshaw et al., 1996c).

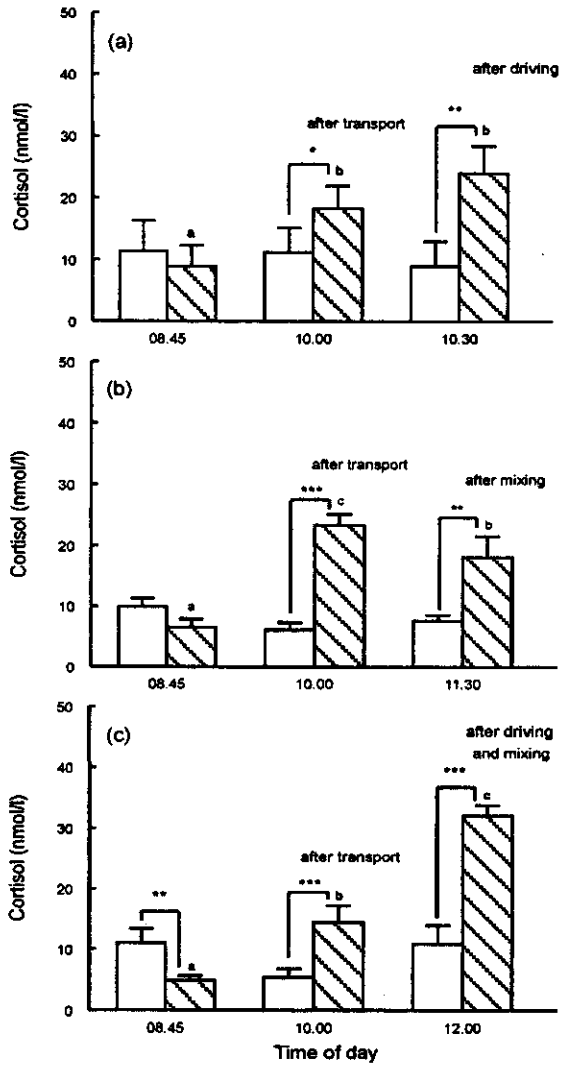

Figure 1. Mean (± SEM) concentration of cortisol before transport, after transport, and after each of treatment (a) driving, (b) mixing and (c) combined driving+mixing. Basal levels in the home pen were measured at the same time of day when pigs were 17 weeks old (controls, open bars) and are compared here with those during the experiment (hatched bars). Means were calculated for each group, and subsequently averaged over group means. A Wilcoxon Signed Rank test was carried out on individual data to 1) test for significant differences between basal (control) and experimental treatments (indicated by asterisks), and 2) test for differences before transport, after transport and after treatment (means lacking a common superscript differ significantly).

After transport, the driving treatment apparently did not lead to any further elevation in concentrations of cortisol. The mean order during driving was not related to social status in the home pen, which agrees with previous results (Blackshaw et al., 1994). Also activity in the home pen did not appear to be related to the order during driving.

After mixing concentrations of cortisol were found to decrease relative to after transport (but were higher than pre-loading levels) which is initially surprising because it is well known that unfamiliar pigs fight which leads to elevated concentrations of cortisol (e.g. Parrott and Misson 1989, Tan and Shackleton 1990). Mixing during transport is also known to be stressful and leads to fighting and elevated concentrations of salivary cortisol (Bradshaw et al., 1996a). However, in the present study, cortisol was not measured during the hour of mixing, and it therefore seems likely that there may have been an increase during the first half hour when fighting was the most intense followed by a subsequent decline as the pigs became exhausted and lay down. Indeed, most fighting was observed to occur in the first half hour after mixing which supports previous findings (Moss, 1978). The frequency and duration of aggressive encounters during mixing were related to level of aggressive behaviour sampled in the home pen which suggests that certain individuals were predisposed to perform aggressive acts regardless of context. In addition, duration of fighting was inversely related to decrease in cortisol, which implies that cortisol response in fighting pigs is either increased or prolonged. Besides the finding that skin damage increase was highest after the mixing treatment, we also found that duration of fighting was positively correlated with increase in skin damage. Thus, fighting during mixing affects cortisol levels and skin damage, in accordance with observations in lairage by Warriss and Brown (1985), Karlsson and Lundström (1992) and Geverink et al. (1996).

The combined treatment resulted in increased concentrations of cortisol which was not predicted by home pen levels of cortisol or increase in cortisol after transport. The various relationships which had been found for the mixing treatment were absent and although total duration of fighting did not differ from the mixing treatment, involvement in fighting was not significantly correlated to aggression shown in the home pen or increase in cortisol levels. These results appear to indicate that driving the pigs before mixing them increases their cortisol response and diminishes individual differences in response. Despite spending the same time fighting in the two situations, skin damage showed a trend to be lower following the combined treatment. Fighting may have been less intense in the combined treatment due to the pre-mixing driving, which may have resulted in the pigs becoming more exhausted.

In conclusion, the combined events of driving and mixing, which are very common in lairage, lead to a greater cortisol response than in the case of each individual treatment. Finally, individual differences shown during mixing were diminished when a period of pre-mixing driving was included.

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Appendix to Chapter 3

Effects of simulated lairage conditions on meat quality

Abstract

The relative effects of the driving, mixing and combined treatment of Chapter 3 on several meat quality variables were studied. Skin damage post-mortem was higher in the mixing treatment but no other differences were detected between treatments. Within the mixing treatment, high concentrations of cortisol after mixing were associated with a higher skin damage score post-mortem and a higher muscle pH at 45 min post-mortem. Together with the results from Chapter 3, which showed that cortisol levels correlated with both fighting and skin damage, this suggests that within groups, individual differences in fighting behaviour may be associated with differences in meat quality.

Introduction

It is widely accepted that preslaughter treatment can influence meat quality. Psychological or physical stress immediately before slaughter may lead to an excessive glycogenolysis with lactate formation, resulting in PSE (pale, soft, exudative) meat (Eikelenboom, 1988). Prolonged stress will lead to low glycogen levels at slaughter, resulting in DFD (dark, firm, dry) meat (Tarrant, 1989). Important events during preslaughter treatment are transport, driving and mixing. Transport by lorry is known to affect meat quality (Lambooy and van Putten, 1993). Driving pigs in lairage is suggested to exacerbate any effects of previous transport and handling on meat quality (Warris et al., 1995). Mixing groups of previously unfamiliar pigs during transport and in lairage has been shown to lead to both more PSE and DFD (Karlsson and Lundström, 1992).

The aims of this study were 1) to compare the relative effects of driving, mixing and a combination of driving and mixing on several meat quality variables and 2) to study individual differences within treatments in meat quality. The test results of this study are only indicative, because immediately after transportation and treatment, pigs were re-loaded and transported again before slaughter. By this time, treatment effects could well have gone. Furthermore, because the time span from the treatment until slaughter varied between treatments, treatment and time from treatment to slaughter were confounded.

Material and Methods

Immediately after the driving, mixing and combined treatment as described in Chapter 3, pigs were once again loaded onto the lorry. They were transported for 30 min to a commercial slaughterhouse where they were unloaded approximately 15 min after arrival. Average time spent in lairage was 20 min during which time the pigs were showered.

After scalding and evisceration, skin damage in the front (cranial to the caudal point of the shoulder), middle and hind region (caudal to the hipbone) was subjectively assessed using a 4-point scale which takes product utilisation into account (Barton-Gade et al., 1996): 1 = no skin damage; 2 = slight skin damage; 3 = skin damage affecting quality; 4 = extreme skin damage with possible rejection of tissue.

Rigor mortis in the m. semimembranosus muscle (SM) on the split carcass was measured 40 min post-mortem using a rigormeter (Sybesma, 1966). The following values were used: 0-6: relaxed; 7-10: more or less in rigor; 11-15: in rigor. pH in the dorsal muscle or m. longissimus dorsi (LM) and the SM were assessed at 45 minutes post-mortem using a pH-meter with an Ingold electrode (Xerolyte, type LOT 406).

A biopsy needle (see Chapter 4) was used to obtain muscle samples (40 to 80 mg) from the LM 5 cm caudal from the last rib 60 min post-mortem for measurements of glycogen. The muscle samples were frozen in liquid nitrogen within 10 sec of sampling and stored at -70°C until analysis. Samples were freeze-dried overnight. Freeze-dried muscle samples were dissected free from connective tissue, blood and fat, and powdered with a pulverizing machine (type MM2, Retsch B.V., Ochten, Netherlands). Five to 10 mg of the powdered muscle tissue was suspended in 200 ul of 0.5 M perchloric acid for the measurement of glycogen. Conversion of glycogen to glucose by amyloglucosidase was conducted (method according to Haagsma et al., 1981). Glucose was extracted by suspension of powdered material in 0.5 M perchloric acid (20 to 40 mg dried tissue/mL of acid). The suspension was centrifuged (10 min, 1500 g) and the clear supernatant neutralized (pH 6.5 to 7.0) with 5.4 M KOH. The total amounts of glucose and G-6-P were measured according to the procedures described in Bergmeyer et al. (1970). Glycogen content was determined after a correction for free glucose and G-6-P already present in the extract.

Statistical analysis

The data were analysed with an analysis of variance model with a factor for experimental group and a factor for treatment (driving, mixing, combined). In addition, for each treatment animals were classified as low or high responders with respect to cortisol levels observed after treatment (see Chapter 3). Within each treatment, behavioural and meat quality parameters were analysed with an analysis of variance model, with a factor on two levels ("low" and "high") for the cortisol classification and a factor for differences between experimental groups. All calculations were performed with the statistical programming language GENSTAT 5 (1993).

Results

Skin damage was higher in pigs from the mixing treatment than for pigs from the other two treatments. The other meat quality variables did not differ between the three treatments (Table 1). However, within the mixing treatment some differences were found. The pigs with high concentrations of cortisol after mixing were those showing a higher carcass skin damage and higher pH values in both SM and LM 45 min post-mortem than pigs with low concentrations of cortisol (Table 2). Within the driving and combined treatment, no such differences were found.

Table 1. Meat quality parameter means (± SEM) for the three treatments

Variable	Treatment		
	Driving	Mixing	Driving + mixing
Skin damage	2.6±0.2[a]	3.6±0.1[b]	3.3±0.1[b]
Rigor mortis	1.8±0.8	1.7±0.5	2.3±0.5
pH45 SM	6.38±0.13	6.47±0.09	6.38±0.09
pH45 LM	6.18±0.12	6.37±0.09	6.26±0.08
Glycogen (μmol/g wet muscle)	28.05±6.37	20.62±4.46	19.71±4.43

[a,b] Means within a row lacking a common superscript differ significantly ($P < 0.05$).

Discussion

The mixing treatment resulted in higher carcass damage post-mortem than the driving or combined treatment. The values were slightly higher than the scores assessed in the live animals in Chapter 3, probably because the bruises were better visible in the scalded and eviscerated carcasses. These bruises may reduce the carcass value because they are still detectable in the processed food (Tarrant, 1989). There were no significant differences between the three treatments regarding any of the other meat quality variables measured. After the treatments, pigs had to be transported to the abattoir and this procedure may have reduced any differences between treatments. However, within the mixing treatment a difference was detected. High concentrations of cortisol after mixing were associated with a higher pH at 45 min in both SM and LM. Together with the results from Chapter 3, which showed that cortisol levels correlated with both fighting and skin damage, this may indicate that these pigs are at risk of developing DFD. This is in agreement with a study by Warriss and Brown (1985), which showed that after

Table 2. Mean values (±SEM) for pigs with high and low concentrations of salivary cortisol after each treatment in relation to various behavioural and meat quality parameters.

	Driving			Mixing			Driving+mixing		
	Cortisol (nmol/l)			Cortisol (nmol/l)			Cortisol (nmol/l)		
	High (n=6) 39.22 ± 5.54	Low (n=7) 11.58 ± 5.13	P	High (n=13) 26.02 ± 3.53	Low (n=13) 9.65 ± 3.52	P	High (n=14) 46.18 ± 2.93	Low (n=12) 15.75 ± 3.17	P
Skin damage	2.8 ± 0.2	2.4 ± 0.2	0.13	3.9 ± 0.2	3.3 ± 0.2	0.01	3.4 ± 0.2	3.3 ± 0.2	0.55
Rigor mortis	2.0 ± 0.5	1.6 ± 0.5	0.54	1.8 ± 0.5	1.8 ± 0.5	1	2.1 ± 0.6	2.5 ± 0.7	0.65
pHSM	6.36 ± 0.19	6.39 ± 0.17	0.89	6.61 ± 0.10	6.32 ± 0.10	0.03	6.38 ± 0.10	6.38 ± 0.11	0.97
pHLM	6.27 ± 0.18	6.11 ± 0.16	0.51	6.53 ± 0.11	6.19 ± 0.11	0.03	6.28 ± 0.11	6.24 ± 0.12	0.81
Glycogen (μmol/g wet muscle)	26.23 ± 6.41	28.70 ± 6.41	0.79	18.34 ± 3.87	22.89 ±4.03	0.42	16.21 ± 4.17	23.07 ± 4.50	0.26

mixing, the amount of carcass damage was related to plasma cortisol levels at sticking, and ultimate muscle pH values. Their pH values at 45 min post-mortem were comparable to our values. A pH value of 6.4 and above may correspond to slow glycolysis (Barton-Gade et al., 1996). It is known that the ultimate pH of meat is among others determined by the amount of glycogen at slaughter (Warriss et al., 1989). It is likely that in pigs with high levels of cortisol, the amount of glycogen was substantially depleted during mixing as a result of muscular activity during fighting. However, concentrations of glycogen 45 min post-mortem were not significantly different between pigs with high and low cortisol levels.

In conclusion, skin damage post-mortem and muscle pH at 45 min post-mortem were related to salivary cortisol levels after mixing, suggesting that individual differences in fighting within groups could be related to differences in ultimate meat quality.

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Chapter 4

The effect of shot biopsy on behaviour, salivary cortisol and heart rate in slaughter pigs

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Abstract

This paper describes behavioural and physiological responses of slaughter pigs to the shot biopsy, a method commonly used to study muscle tissue processes and predict meat quality. From 10 23-wk old gilts, one sample from the longissimus muscle was obtained using a cannula connected to a captive bolt. Ten other gilts were used as a control and received a sham shot. One week later, a second shot biopsy was taken from the experimental group. Behavioural and salivary cortisol responses to both biopsies were the same. All pigs flinched in response to the biopsies. Salivary cortisol was increased after 15 min. In both tests heart rate increased significantly in response to the presence of the technician. In response to the first biopsy heart rate increased significantly but not in response to the second biopsy. The experimental pigs showed a decrease in initiating contact with the technician in the second test. It is concluded that shot biopsy had a significant acute effect on behaviour and physiology. Therefore, the usefulness of the technique in studies on the relation between pre-slaughter stress and meat quality is limited.

Introduction

Porcine meat quality can be predicted to a certain extent by studying properties of the skeletal muscle in live pigs (Lahucky, 1987). A commonly used method to collect muscle samples ante-mortem is the so-called shot biopsy (Lahucky et al., 1982; Wegner and Ender, 1990). The shot biopsy is carried out with a specially adapted common slaughter pistol with a cannula on the top. The muscle samples are collected by a "shot-out" on live pigs. Samples of up to 1.5 g in dependence on animal size can be obtained. Several studies report that pigs subjected to the shot biopsy do not need to be restrained nor anaesthetized (Lahucky, 1987; Schöberlein, 1989), and wound healing and muscle tissue regeneration seem to proceed without complications (Schöberlein, 1989).

So far, the shot biopsy method has been applied in studies on effects of e.g. sex (Talmant et al., 1989), or genetic background (Cheah et al., 1993) on meat quality. However, besides genetic factors, also preslaughter treatment will influence meat quality (Tarrant, 1989). Various studies are therefore concerned with the relationship between preslaughter stressors and meat quality (e.g. Grandin, 1980; Warris and Brown, 1985; Warris et al., 1995). In such studies, important variables that quantify the effect of the stressor on the live animal are behaviour, cortisol levels and heart rate. Obviously, shot biopsy variables could provide useful information about the

effects of pre-slaughter stress on meat quality. However, a prerequisite is that the shot biopsy itself does not have an effect on the behavioural and physiological variables. Although most authors claim that the shot biopsy method ensures minimal stress (Schöberlein, 1989; Wegner and Ender, 1990; Fernandez et al., 1992), there seems to be no experimental evidence available. Therefore, the present experiment was conducted to estimate the short-term effects of a shot biopsy on behaviour, salivary cortisol levels and heart rate in slaughter pigs. The same procedure was repeated one week later to test whether responses to a second exposure differed from those to the first one.

Material and Methods

Subjects and housing

Subjects were 20 crossbred gilts (Great Yorkshire x (Great Yorkshire x Dutch Landrace)). They were kept single-housed in pens with a fully slatted concrete floor (1.55 x 1.70 m) in a room that was enclosed and temperature-controlled. Main artificial lights were on from 0600 until 1800. Animals were given ad libitum access to water and feed. The average live weight at the age of 23 weeks was 100 kg.

Experimental design

Pigs were assigned to two treatment groups, a biopsy group (n = 10) and a control group (n = 10).

Test 1. At 23 weeks of age, one sample from the LM was obtained from all animals in the biopsy group. The biopsy device was similar to the devices used in other studies (Lahucky et al., 1982; Schöberlein, 1989; Fernandez et al., 1992) and consisted of a home-built cannula connected by a bayonet-catch to a specially adapted common captive bolt (Goldhaser Schiessapparat, Brutmachine Jahn. GmbH, Hammelburg, Germany). The stainless-steel cannula had a length of 6.5 cm and was 0.7 cm in cross section (Fig. 1). A thin steel blade cut the sample during cannula withdrawal. The procedure of taking a biopsy was as follows. The technician entered the pen and knelt in the middle. The pig was allowed to move free in the pen during the sampling process. The technician examined the pig's back to locate the last rib, and made a pencil mark 5 cm caudal of the last rib, around 5 cm apart from the dorsal line, on the right side (Fig. 2). Then he pressed the biopsy device on this mark and applied the biopsy shot. This procedure took 1 min. After applying the shot, the technician immediately left the pen. The procedure for animals in the control group

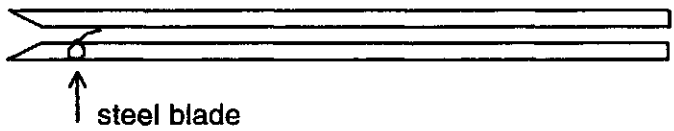

Fig. 1. Cross-section of the cannula. A thin steel blad cuts the muscle sample during cannula withdrawal.

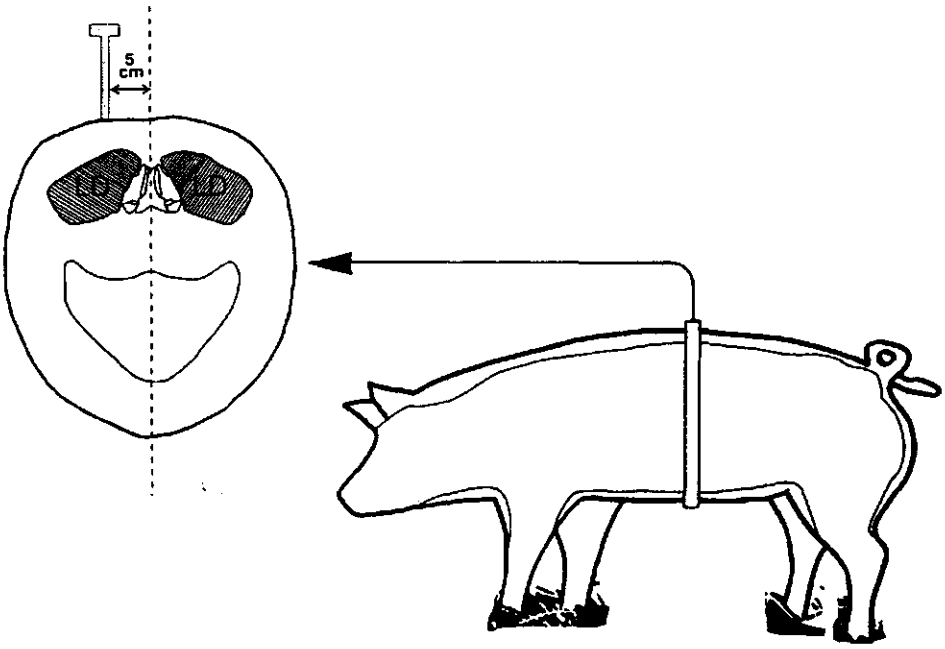

Fig. 2. Cross section 5 cm caudal of the last rib. The place where the biopsy shot is applied is indicated.

was identical, except for the actual shot itself; the biopsy device was only held on the pencil mark and no sample was taken ('sham shot'). Sampling was performed during five days between 1415 and 1530 (Table 1). The afternoon period was chosen because cortisol levels are believed to be more stable then (Barnett et al., 1981).

Test 2. The subsequent week, pigs in the biopsy group were subjected to a procedure identical to that in test 1, but with the sample taken from the left side. The same design as in test 1 was used (Table 1) but only data from the biopsy group were used in the analysis.

Behaviour

The behavioural responses of the animals during the procedure were recorded on videotape. Behaviours scored from videotape were: frequency of initiating contact with the technician (nosing, chewing overalls), ambulation during presence of the technician and during 5 min after the biopsy shot, and acute responses (flinch, vocalize, rub back against wall) to the biopsy. In order to score ambulation, the pen was divided in two imaginary sections and the number of times the pig crossed the intersection was scored.

Table 1. Treatment schedule for 10 pigs receiving a biopsy shot and 10 pigs receiving a sham shot

Day	Time of day			
	1415	1440	1505	1530
1	Sham	Biopsy	Sham	Biopsy
2	Biopsy	Sham	Biopsy	Sham
3	Sham	Biopsy	Sham	Biopsy
4	Biopsy	Sham	Biopsy	Sham
5	Sham	Biopsy	Sham	Biopsy

Salivary cortisol

The use of saliva rather than blood for sampling cortisol has been validated in pigs (Parrott and Misson, 1989; Parrott et al., 1989). Saliva samples for cortisol assessment were taken by the experimenter 30 and 4 min before the biopsy, and 5, 15, 30, 60 and 90 min after the biopsy. Saliva samples were collected by allowing the pig to chew on two cotton buds until they were thoroughly moistened. It took on average 1 min to take a saliva sample. The pigs were accustomed to this procedure during the preceding week. The cotton buds were stored in test tubes, kept on ice and subsequently centrifuged at 5000 *g* for 5 min to remove the saliva which was stored at -20°C until assay. Cortisol concentration was measured using a commercial RIA kit (Coat-a-Count, DPC, The Netherlands) modified for pig salivary cortisol (Ruis et al., 1997).

Heart rate

Heart rate was recorded with Polar Sport Testers (Polar Electro OY, Finland). The equipment consisted of an electrode belt with built-in transmitter and a wrist-watch receiver. The receiver had a memory function and stored data from the transmitter, averaging heart rate over 5-s intervals. After the first saliva sample at t = -30 min was taken, the experimenter fitted the electrode belt around the thorax of the pig caudal to the forelimbs. The experimenter wore boots and overalls of the same color as the technician. The receiver was fastened around the belt and positioned on the dorsal midline. The pigs were accustomed to this procedure during the preceding week. Heart rate data were collected from 15 min before the biopsy shot until 90 min after the biopsy shot. Afterwards, data were downloaded via a Polar Interface (Polar Electro Oy, Finland) onto a PC.

In order to analyze heart rate, mean heart rates were calculated from running means for the following periods (with time of shot set at t = 0 min):

- start: t = -15.0 min until t = -4.0 min. At t = -4.0 min, the first saliva sample was taken.
- saliva samples: each min during which saliva was sampled, using each previous min as a reference value.
- technician present: t = -1.0 min until t = 0 min, using the previous min as a reference value.
- after shot: each 5 s from t = 0 onwards, until heart rate was not different anymore

from the 5-s period before the shot.

- after technician + shot: each min from t = 0 onwards, until heart rate was not different anymore from the start period, the second half hour (t = 31.0 min until t = 60.0 min), and third half hour (t = 61 min until t = 90.0 min).

Statistical analysis

Data were analysed with an analysis of variance model. Factors in the model were day of experiment, sequence of usage during the day of experiment (first, second, third or last) and treatment (biopsy shot, sham shot). No significant interactions were found between sequence of usage and treatment and therefore the model was reduced to main effects only.

Fisher's exact test was used to analyse differences in presence or absence of acute behavioural responses between the biopsy and sham shot treatment. Sign test was used to compare differences in presence or absence of acute behavioural responses between the first and second biopsy shot. Within-animal differences between other behavioural parameters in response to the first and second biopsy shot were analysed with Wilcoxon's signed-rank test (Conover, 1980). Within-animal changes in cortisol levels and heart rate were analysed with a paired t-test.

All calculations were performed with the statistical programming language Genstat 5 (1993).

Results

Test 1

Behaviour. In the biopsy group, 70% of the pigs vocalized at the moment the biopsy was taken but in the control group none of the pigs vocalized in response to the sham shot ($P < .01$). All pigs in the biopsy group flinched in response to the biopsy compared to none in the control group ($P < .001$). Two pigs in the biopsy group rubbed their backs several times against the side of the pen within 5 min after the biopsy was taken, compared to none in the control group ($P = 0.47$). There were no differences between biopsy and sham shot group in the frequency of initiating contact with the technician (4.4 ± 1.0 vs 1.9 ± 1.2, ns), ambulation in presence of the technician ($5.5 \pm .7$ vs $3.9 \pm .8$, ns), or ambulation during 5 min after the shot (6.2 ± 1.0 vs 8.8 ± 1.2, ns). It was observed (not measured) that ambulation of the pigs was higher during the presence of the technician than during the previous period.

Cortisol. Mean levels of salivary cortisol previous to the biopsy and sham shot

(averaged over t = -30 min and t = -4 min) were not different between biopsy group (.79 ± .14 ng/ml) and control group (.98 ± .13 ng/ml) (Fig. 3). Fifteen min after the biopsy shot, cortisol levels had increased with 1.24 ± .48 ng/ml ($P < .05$). Sham shot did not increase cortisol levels (increase .002 ± .17 ng/ml 15 min after sham shot, ns). However, only at t = 30 min, cortisol levels showed a trend to be higher in the biopsy group compared to the control group (1.63 ± .62 vs .72 ± .12 ng/ml, $P < 0.1$). Mean cortisol levels averaged over the five samples following the biopsy shot were higher, though not significantly, in the biopsy group (1.27 ± .44 ng/ml) compared with the control group (.72 ± .31 ng/ml).

Heart rate. Mean heart rate in the start period was 122.6 ± 3.9 bpm in the biopsy group and 120.5 ± 4.7 bpm in the control group. Heart rate was increased during the min the technician was present in the pen (compared to the previous min) in both biopsy group (increase with 32.3 ± 4.8 bpm, $P < .001$) and control group (22.1 ± 4.5 bpm, $P < .001$) (Fig. 4 and 5). The increase in the biopsy group was not significantly higher than in the control group. Shot biopsy further increased mean heart rate with 21.8 ± 5.8 bpm ($P < .01$). The increase to the biopsy lasted 30 sec.

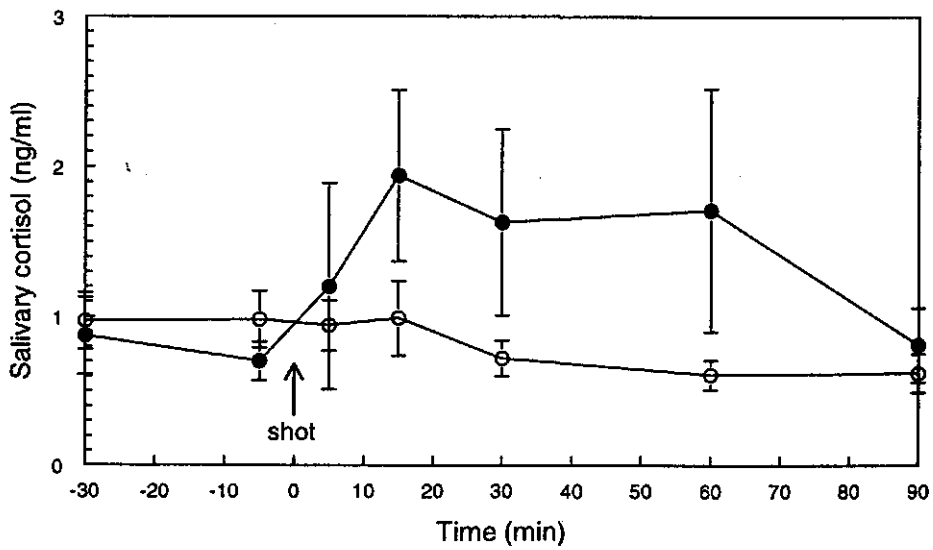

Fig. 3. Mean (± SE) salivary cortisol levels in gilts subjected to a biopsy shot (●, n=10) and a sham shot (○, control, n=10).

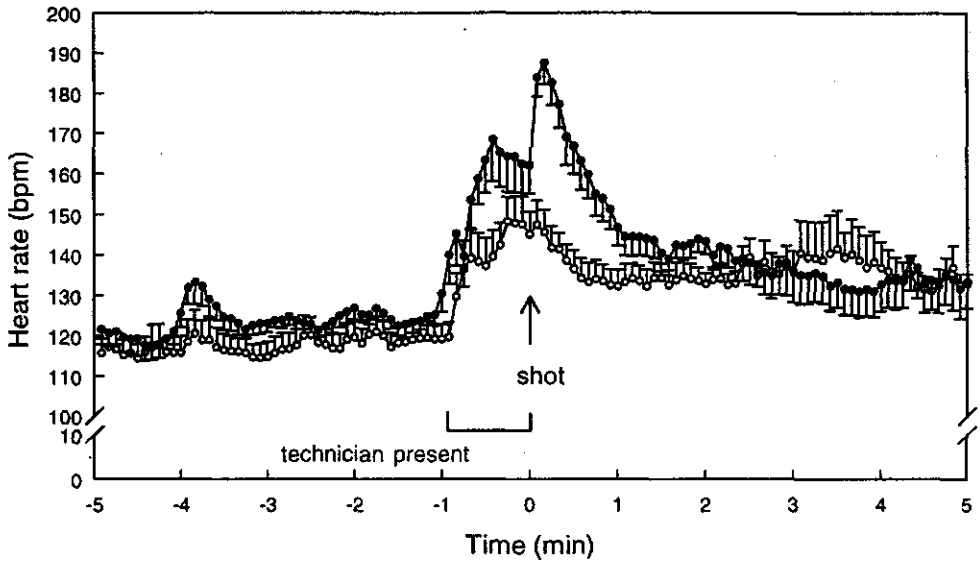

Fig. 4. Mean (± SE) heart rate in gilts subjected to a biopsy shot (●, n=10) and a sham shot (○, control, n=10), from 5 min before until 5 min after the shot.

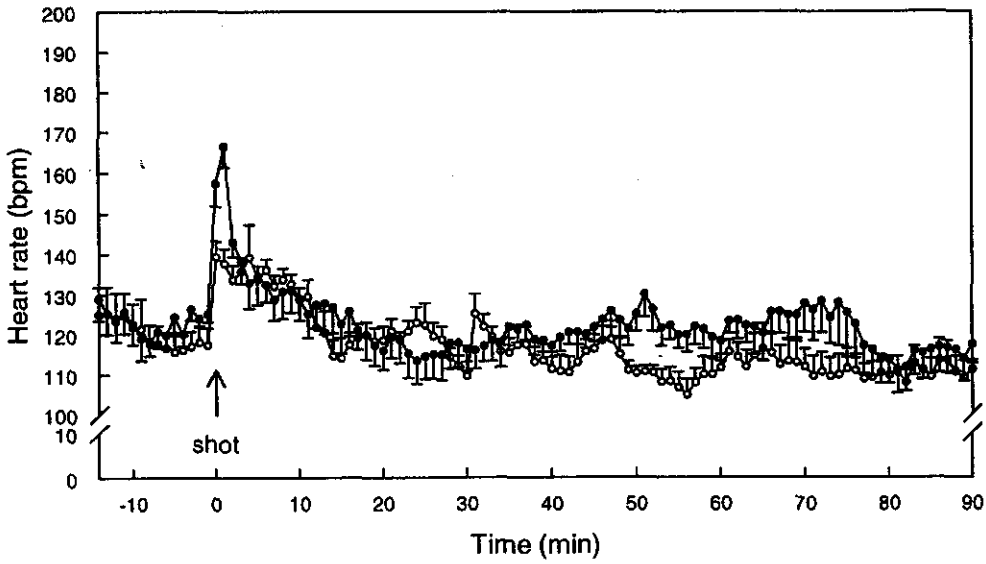

Fig. 5. Mean (± SE) heart rate in gilts subjected to a biopsy shot (●, n=10) and a sham shot (○, control, n=10), from 15 min before until 90 min after the shot.

In the 5th min after the biopsy shot heart rate was not different anymore from heart rate during the start period. In the control group, heart rate was not increased by the sham shot (increase of 2.4 ± 2.6 bpm, ns). In the 5th min after the sham shot, heart rate was not different anymore from heart rate during the start period.

Sampling saliva 4 min before the shot was associated with a slight increase in heart rate in the biopsy group (6.4 ± 2.6 bpm, $P < .05$) but not in the control group (.76 ± 1.6 bpm, ns). Sampling saliva after the biopsy and sham shot did not affect heart rate.

Test 2

Behaviour. Acute responses to the second biopsy were not different from those to the first one. Ninety percent of the animals vocalized at the moment the second biopsy was taken, and all animals flinched. In addition, three pigs (different individuals from those in test 1) rubbed their back against the side of the pen within 5 min after the biopsy shot. The frequency of initiating contact with the technician had decreased (test 1: 4.4 ± 1.0; test 2: 1.5 ± .4; $P < .05$), but there was no difference in ambulation in presence of the technician (test 1: 5.5 ± .7, test 2: 6.7 ± .9), or ambulation during 5 min after the biopsy shot (test 1: 6.2 ± 1.0, test 2: 7.6 ± .8).

Cortisol. Basal levels of cortisol before the biopsy shot (.94 ± .21 ng/ml), cortisol increase 15 min after the biopsy shot (1.16 ± .37 ng/ml) and levels over the 90 min period following the biopsy shot (1.53 ± .43 ng/ml) were not significantly different from those in test 1 (Fig. 6).

Heart rate. Mean heart rate in the start period (113.4 ± 4.4 bpm) and heart rate increase during the min the technician was present in the pen (32.1 ± 9.4 bpm) were not different from those in test 1 (Fig. 7 and 8). Mean heart rate did not increase in response to the biopsy (9.8 ± 10.1 bpm, ns). In the 7th min after the biopsy shot heart rate had reached start levels again. Sampling saliva 30 min after the biopsy shot was associated with an increase in heart rate with 10.8 ± 4.4 bpm ($P < .05$), while sampling saliva at other times had no effect on heart rate. Mean heart rate was lower after the second biopsy than after the first biopsy in the period 30-60 min (106.8 ± 4.0 vs 120.9 ± 3.1 bpm; $P < .01$) and 60-90 min (108.5 ± 4.8 vs 120.1 ± 5.3 bpm; $P < .05$) than heart rate after the first biopsy, but when these values were corrected for start levels the differences were not significant anymore (data not shown).

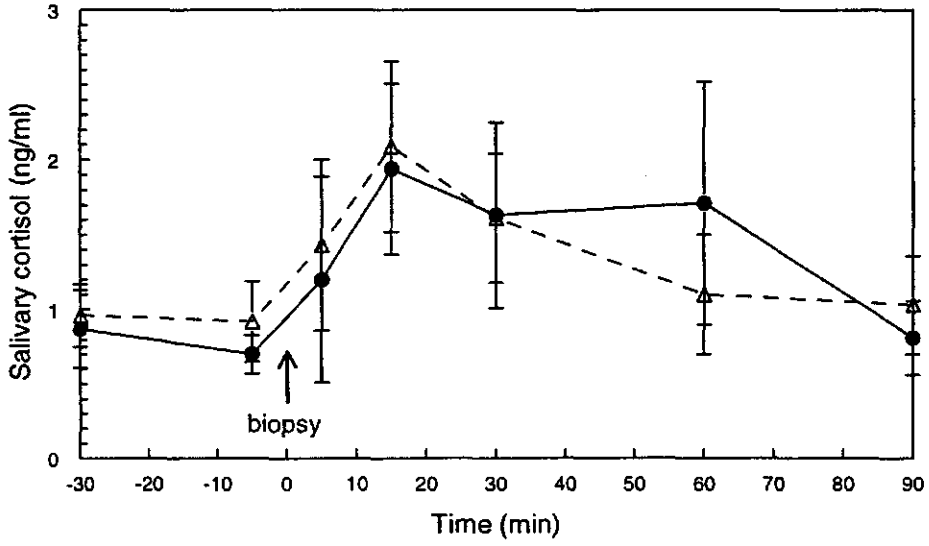

Fig. 6. Mean (± SE) salivary cortisol levels in gilts (n=10) in response to the first (●) and the second (Δ) biopsy shot one week later.

Discussion

This experiment showed that shot biopsy induced acute behavioural, salivary cortisol and heart rate responses in slaughter pigs, although salivary cortisol responses were not very pronounced and heart rate response to a second biopsy shot was absent. The pigs showed several behavioural responses to both biopsies. All animals flinched and most animals vocalized. In addition, some of them rubbed their backs against the side of the pen. These behaviours may be generally described as specific pain behaviours (Molony and Kent, 1997). Furthermore, the decrease in frequency of contact with the technician test suggests that the first biopsy was perceived as aversive. Various other studies have shown that the imposition of aversive handling procedures resulted in avoidance of humans (Gonyou et al., 1986; Hemsworth et al., 1986; Hemsworth and Barnett, 1991).

The biopsy shot also affected cortisol levels. In the control group, cortisol levels remained at their basal levels in response to the sham shot. In contrast, experimental pigs showed significantly increased cortisol levels 15 min after the biopsy shot in both test 1 and 2, although post-biopsy levels in test 1 were not significantly different from

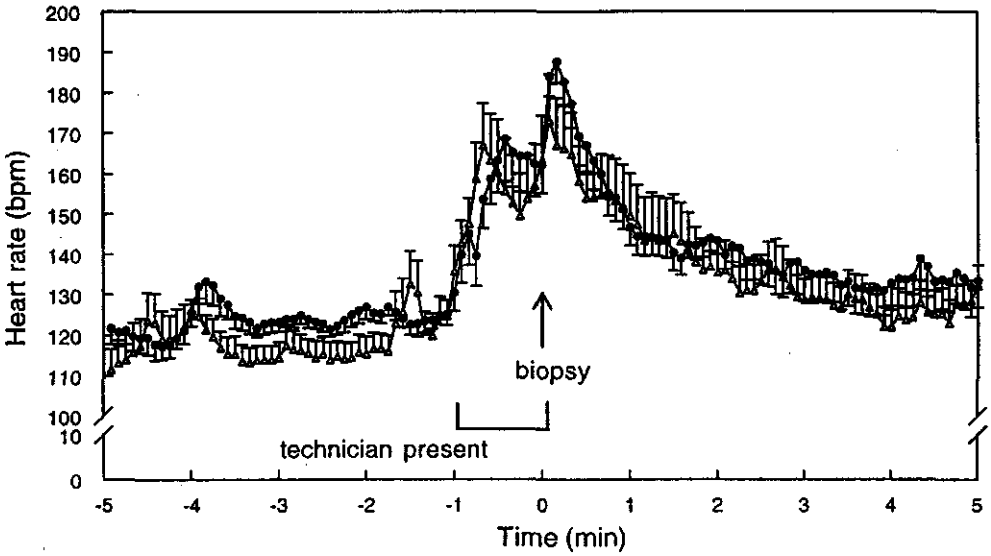

Fig. 7. Mean (± SE) heart rate in gilts (n=10) 5 min before until 5 min after the first (●) and the second (Δ) biopsy shot one week later.

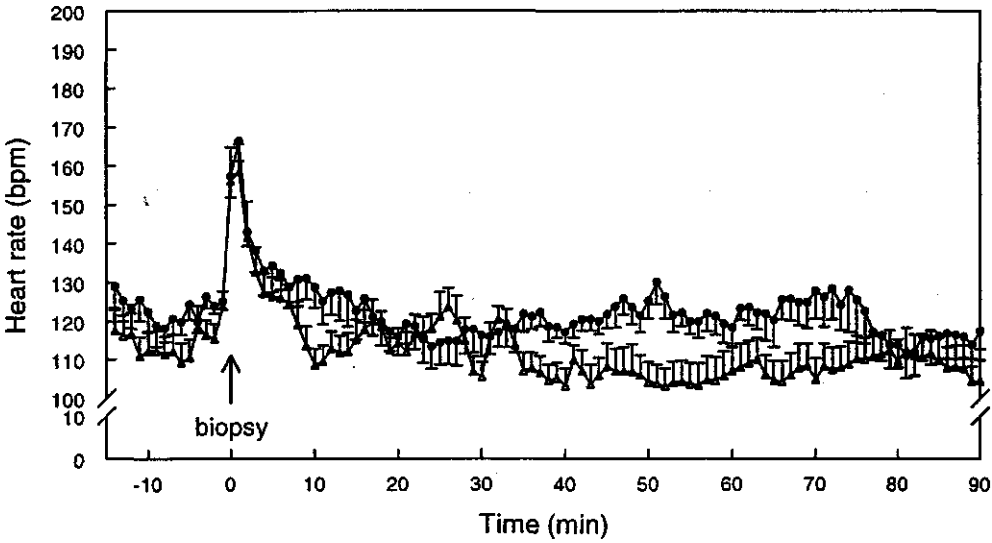

Fig. 8. Mean (± SE) heart rate in gilts (n=10) 15 min before until 90 min after the first (●) and the second (Δ) biopsy shot one week later.

control values. This could be attributed to the large inter-individual differences in cortisol response to the biopsy shot, while variation of cortisol levels in the control group was relatively small. The cortisol increase in response to the second biopsy was similar to the increase in response to the first biopsy. From other studies it is well-known that an animal's stress response decrements over time despite repetitive exposure to identical stressors (Natelson et al., 1988; Klemcke, 1994), but glucocorticoids are known to be slower in adaption than e.g. adrenaline and noradrenaline (de Boer et al., 1988). Therefore, if habituation of cortisol levels to biopsy occurs, it may take more than two trials to achieve this.

Heart rate was increased significantly in both the biopsy and the control group during the presence of the technician. It was observed that the pigs showed an increased physical activity in response to the actions of the technician. As heart rate is thought to increase with locomotion in mammals (Baudinette, 1978; Smith et al., 1993), the observed increase in heart rate is likely to be partly due to an increased physical activity. In other studies, it has been demonstrated that an aversive treatment can result in a bradycardiac response in anticipation to a second treatment, for example in foot-shocked rats (Korte, 1990), but heart rate increase prior to the second biopsy did not differ from increase prior to the first biopsy.

The first shot biopsy caused a short-term tachycardia, which in combination with the flinch response suggests an arousal of the sympathetic nervous system. The second biopsy did not increase heart rate anymore, although the withdrawal reflex was still present. It is possible that due to the predictability of the biopsy, the response was diminished. It has been shown that the ability of an animal to control a stressful environmental event and predict the occurrence of the event seem to be important variables influencing the stress response (see Gray, 1987). Furthermore, other authors have suggested that the opportunity to interact with the experimenter may be a rewarding component of an aversive treatment for animals that are housed under barren conditions with low levels of stimuli, and this may diminish a stress response (Hemsworth et al., 1996). However, our behavioural data do not seem to support this.

Thus, a shot biopsy induces behavioural, salivary cortisol and heart rate changes, with the behavioural data suggesting aversiveness. Therefore, the usefulness of the technique in studies on the relation between pre-slaughter stress and meat quality seems to be limited.

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Chapter 5

Responses of slaughter pigs to transport and lairage sounds

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Abstract

The behavioural and physiological responses of pigs to transport and subsequent exposure to slaughterhouse sounds were examined. Fourty-three groups of four slaughter pigs were separately loaded onto a lorry and transported for 25 min. Another 41 groups were loaded onto the lorry which then remained stationary for 25 min. Following unloading pigs were moved to a race with a length of 15 m and a width of 1.5 m. Either one of the following sounds was played at 85 dB(A) for 10 min: Pigs in front of the restrainer, Machines in lairage, White noise, or Control (no sound). Pigs exposed to the Machines and White noise treatment spent significantly more time close to their groupmates compared with Control pigs, with pigs subjected to the Pig sound being intermediate. Transported pigs spent less time exploring the race and were less active than pigs from the stationary lorry. Heart rate was higher during transport than during the stationary period. In contrast, during unloading, the sound exposure period and the post-sound period, heart rate was lower in the transported groups. Heart rate did not significantly differ between sound treatments. Salivary cortisol concentrations were significantly higher after transport than after the stationary period and remained higher for transported pigs after the sound exposure period. Cortisol levels did not differ significantly between sound treatments. It is tentatively suggested that social support from conspecifics may protect pigs from potentially adverse effects of exposure to lairage sounds.

Introduction

In lairage, pigs are often observed to be reluctant to move. Apart from failures in design (Grandin, 1990; Barton-Gade et al., 1992; Warris et al., 1992), pigs might perceive other environmental aspects as aversive. It has been suggested that noise may be one of the disturbing factors (Grandin, 1983).

There is experimental evidence that farm animals respond to noise in laboratory settings, and data suggest noise may be aversive. For example, calves showed an increase in plasma adrenalin and cortisol when exposed for 30 min to the 96 dB noise of a transport simulator (Agnes et al., 1990). Exposure of pigs to simulated transport (noise and vibration) led to an increase in plasma vasopressin (Forsling et al., 1984). Stephens et al. (1985) taught pigs to press a switch panel to turn off a transport simulator which produced vibration and noise. All pigs responded behaviourally to terminate the simulator. When the vibration component was switched off the pigs continued to make the response to switch the noise component off, though with a lower frequency. A recent

report showed that 1-month old piglets respond with an increase in heart rate and ambulation score when exposed to 15 min of artificially generated sounds (Talling et al., 1996).

The studies mentioned above each tested one type of noise. The sound in a slaughterhouse consists of various components, with the most important contributions coming from the squeals of pigs driven to the restrainer, and the sound of machines that process the carcasses. Of interest is whether pigs find certain components more aversive than others. This can be tested if pigs are able to distinguish between recorded sounds during playback. Several studies by others (Weary et al., 1995; Talling et al., 1996) suggest that this is the case. Sows respond to a greater extent to playback of piglet isolation calls than to playback of white noise (Weary et al., 1995). Significant differences in heart rate and behaviour were observed in 1-month old pigs when exposed to several real sounds (Talling et al., 1996).

The aim of the current experiment was to study whether pigs show differences in behavioural and physiological responses to two components of slaughterhouse sound, i.e. squeals of pigs driven to the restrainer, and the sound of machines. The influence of prior transportion on these responses was also studied.

Materials and methods

Animals and Housing

Subjects were 336 25-week old pigs (Great Yorkshire x (Great Yorkshire x Dutch Landrace), weighing approximately 105 kg). They had been housed in 84 groups of 4 (2 gilts and 2 barrows) from the age of 10 weeks onwards. They were housed in pens (1.8 x 1.6 m) with fully slatted concrete floors. Animals were given ad libitum access to feed and water. Feed was withheld from 06.00 h on the day of the experiment. The pigs were not tested individually but groupwise, because under common transport and lairage conditions pigs are not handled individually.

Materials

Measurements and recordings of sounds of pigs and machines were performed next to the restrainer in the lairage of a large Dutch slaughterhouse (number of pigs slaughtered per hour was 600). The total sound pressure level in dB(A) was measured with a sound level meter (type 2219, Brüel & Kjaer Benelux, Nieuwegein, The Netherlands). The squeals of pigs near the restrainer reached levels of 100-112 dB(A). The machines used for processing the carcasses produced a sound pressure level of 80-85

dB(A). Both sounds were recorded with a microphone (Sennheiser MD 441/421, Sennheiser Nederland, Almere, The Netherlands) connected to a DAT-recorder (Sony TCD-D3, Sony Nederland, Badhoevedorp, The Netherlands). In addition to these sounds, white noise of frequency range 200-20.000 Hz was generated by a sound power source. White noise was chosen as a reference sound because of its absence of biological content and constant sound pressure level.

In an empty pig house, unfamiliar to the experimental pigs, a race of 15 m length and 1.5 m width was created with 1.0 m high hard board walls. Two m from the start of the race, a gate was placed to create a start box where the pigs could be assembled before and after the sound exposure period. Playback of the recorded sounds was presented from a sound recorder (Philips F6121, Philips Electronics, Eindhoven, the Netherlands). An amplifier (LBH 0125, frequency response ± 3 dB(A) over the range 60 - 16 000 Hz, Philips Electronics, Eindhoven, The Netherlands) was used to amplify all auditory stimuli. The sound stimuli were transmitted into the experimental room via a loudspeaker (LBC 3057, Philips Electronics, Eindhoven, the Netherlands) placed at the start of the race at a height of 2 m and directed towards the floor at an angle of 45 degrees. The sounds of pigs, machines or white noise were played for 10 min at 85 dB(A) (measured at a height of 80 cm and at a distance of 1 m horizontally from the loudspeaker). The sound level decreased towards the end of the race to 75-80 dB(A). In the control treatment, no sound was played and sound pressure level was 30 dB(A).

Experimental design

The basis for the experimental design was a combination of two cyclic designs. Cyclic designs are incomplete block designs generated by cyclic development of a suitably chosen initial block. It is considered an easy, flexible approach for generating designs with a high degree of balance. Cyclic designs for 8 and 16 experimental days were combined (see John et al., 1972; schemes B3 and B4). Three groups were treated per day (at 10.00 h, 12.30 h and 15.00 h). This yielded a design with 24 blocks (experimental days), each of size 3, and 8 treatment combinations (transport or no transport x 4 sound treatments). The final design was a modification of the basic design, adding 4 additional experimental days. The number of groups assigned to each treatment ranged from 9 to 13.

Procedures

Treatment of a group of pigs was as follows. At the start of the session (t = 0 min), saliva samples were taken in the home pen. Immediately afterwards, heart rate meters were strapped on. After 45 min, a second saliva sample from each pig was taken. At t = 50 min, the experimental group was loaded onto a lorry. When the group was assigned to a transport treatment, it was transported for 25 min (from t = 55 until t = 80 min). A stationary treatment group was slowly transported (20 km/h) to the experimental building, which took 1 min, and then remained for 24 min in the stationary vehicle. With this set-up, the effects of transportation itself could be studied, without the relative effects of loading and the novelty of the lorry environment.

At t = 80 min, a third saliva sample was taken. At t = 85 min, the pigs were unloaded and moved to the experimental race (distance 12 m). After 3 min to allow exploration of the entire race, the group was moved to the start of the race and the gate of the start box was closed. At t = 90 min, the gate was opened and simultaneously, the sound playback was started. After 10 min, the playback was stopped and the pigs were moved to the start box, where saliva samples were taken at t = 105 and t = 110 min, respectively.

Heart rate. Heart rate was recorded with Polar Sport Testers (Polar Electro OY, Finland). The electrode belt with built-in transmitter was fitted around the thorax of the pig caudal to the forelimbs. The receiver was put in a small box for protection, and positioned on the belt on the dorsal midline. The receiver had a memory function and stored data from the transmitter, averaging heart rate over 5-s intervals. Medical tape (Tesa Band Universal 50 mm, ES Tapes, Hilversum, The Netherlands) was fitted around the belt and receiver for protection. Fitting the heart rate meters was done in the home pen to prevent isolation stress, and took about 5 min for each pig. Average heart rates were recorded from running means for the time periods of each event as mentioned above, with the exception of the home pen period, where only the running means from the last 15 min were used. In addition, regression coefficients were calculated from running means for the 10 min sound period.

Cortisol. Cortisol in saliva is essentially in the free form, providing a good indication of levels of free biologically active cortisol circulating in blood plasma (Kirschbaum and Hellhammer, 1989; Parrott and Misson, 1989; Parrott et al., 1989). Saliva samples for cortisol assessment were taken at several times during the experiment (see above). The pigs were familiar with this sampling procedure, which was performed previously at the age of 14 and 21 weeks. Saliva samples were collected by allowing the

pig to chew on two cotton buds (Hartmann, Nijmegen, The Netherlands) until they were thoroughly moistened. It took on average 1 min to take a saliva sample. The cotton buds were stored in test tubes (Sarstedt, Etten-Leur, The Netherlands) kept on ice and subsequently centrifuged at 4000 *g* for 5 min to extract the saliva which was stored at -20°C until assay. Cortisol concentration was measured using a commercial RIA kit (Coat-a-Count, Diagnostic Products Corporation, Apeldoorn, The Netherlands) modified for pig salivary cortisol (Ruis et al., 1997).

Behaviour. The behaviour of the pigs during the sound exposure period were recorded on video tape through two cameras mounted at the walls, one at the start of the race and one halfway the race. Behaviours were scored from video tape by using behaviour analysis software The Observer 3.0 (Noldus et al., 1997). The race was divided in four imaginary sections of 3.75 m length each, and ambulation score of each pig was measured by counting how many sections the pig entered. The duration of time spent in the two front and the two hind sections was calculated. Also the time spent with all group-mates in the same section was calculated. Duration of standing, lying or sitting, and exploration (sniffing, rooting) of floor and walls was also scored.

Statistical analysis

Variables were analysed with an analysis of variance model. Group means were analysed. Factors in the analysis were 'day of experiment', 'time of day' (10.00 h, 12.30 h, 15.00 h), 'transport' (transport, stationary) and 'sound' (control, pigs, machines, white noise). The model included main effects and the interaction between 'time of day', 'transport' and 'sound'. A paired t-test was used to test differences in heart rate and cortisol concentrations within groups. All calculations were performed with the statistical programming language Genstat 5 (Genstat 5 Committee, 1993).

Results

Because none of the interaction terms in the analysis of variance were significant, all models were reduced to main effects only. Heart rate data are shown in Fig. 1 and Table 1. Heart rate during loading was significantly higher than during previous saliva sampling ($P < 0.001$). During the lorry period, heart rate was significantly higher in the transported groups than in the stationary groups. Unloading significantly increased heart rate in both stationary ($P < 0.001$) and transported pigs ($P < 0.001$). After the transport period, mean heart rate in the transported groups was lower than in the stationary groups.

Table 1. Mean values and sem's for heart rate in each of the transport x sound treatments

	Sound treatment								Treatment effects	
	Control		Pigs		Machines		White noise		Transport	Sound
Time period	Stationary	Transport	Stationary	Transport	Stationary	Transport	Stationary	Transport	P <	P <
Lorry	120.7 ± 2.1[a]	133.5 ± 2.2[b]	121.9 ± 2.2[a]	134.8 ± 2.0[b]	119.9 ± 2.4[a]	132.7 ± 2.4[b]	120.5 ± 2.3[a]	133.4 ± 2.3[b]	0.001	ns
Saliva sample 3	119.7 ± 2.0[b]	116.0 ± 2.1[a]	124.9 ± 2.1[c]	121.2 ± 2.0[b]	120.7 ± 2.3[b]	117.0 ± 2.3[a]	124.9 ± 2.2[c]	121.2 ± 2.2[b]	0.05	0.1
Unloading	162.5 ± 2.9[a]	151.6 ± 3.0[b]	164.6 ± 3.0[a]	153.7 ± 2.8[b]	164.7 ± 3.3[a]	153.8 ± 3.2[b]	168.1 ± 3.2[a]	157.2 ± 3.3[b]	0.001	ns
Δ unloading*	42.8 ± 2.6[a]	35.6 ± 2.7[bc]	39.7 ± 2.8[ab]	32.5 ± 2.5[c]	43.9 ± 3.0[a]	36.8 ± 2.9[bc]	43.2 ± 2.8[a]	36.0 ± 2.9[bc]	0.001	ns
Sound period	160.9 ± 3.5[a]	151.2 ± 4.1[bc]	161.9 ± 3.0[a]	155.8 ± 3.7[b]	153.2 ± 6.7[ab]	145.0 ± 4.2[c]	163.7 ± 5.8[a]	150.6 ± 4.1[bc]	0.01	ns
Regression coefficient†	-0.42 ± 0.65[a]	-1.89 ± 0.69[bc]	0.44 ± 0.68[a]	-1.04 ± 0.65[bc]	-0.53 ± 0.75[ab]	-2.00 ± 0.75[c]	-0.20 ± 0.73[a]	-1.68 ± 0.73[bc]	0.01	ns
Post-sound	144.2 ± 3.1[a]	135.4 ± 3.3[b]	144.0 ± 3.2[a]	135.2 ± 3.0[b]	143.5 ± 3.5[a]	134.7 ± 3.4[b]	145.1 ± 3.4[a]	136.3 ± 3.4[b]	0.001	ns
Saliva sample 4,5	133.3 ± 2.1[a]	127.0 ± 2.2[bc]	134.1 ± 2.2[a]	127.8 ± 2.1[bc]	130.7 ± 2.5[ab]	124.5 ± 2.4[c]	135.5 ± 2.4[a]	124.8 ± 3.9[bc]	0.001	ns

[a,b] Means within a row lacking a common superscript differ significantly ($p < 0.05$)

* Change in heart rate was calculated subtracting mean heart rate during saliva sample 3 from mean heart rate during unloading

† Regression coefficients were calculated from 5-s running means for the 10 min sound period

There was no effect of sound treatment on mean heart rate or regression coefficient during sound exposure.

Cortisol concentrations at t = 0 min (sample 1) and t = 45 min (sample 2) were not significantly different between treatment groups, but concentrations at t = 45 min had significantly increased within all treatment groups (Fig. 2a and 2b). Subsequently, both the transport and the stationary treatment further increased cortisol concentrations, with the concentrations after transport being significantly higher than after the stationary period ($P < 0.001$). There was no difference between sound treatments in cortisol concentrations 5 and 10 min after the sound period, but concentrations in the transported groups remained significantly higher ($P < 0.001$).

Behavioural results are shown in Table 2. Pigs that were transported had a significantly lower ambulation score during the sound period than pigs in the stationary treatment. Transported pigs showed a trend to spend more time lying, and spent significantly less time exploring the race. Pigs in the Machines and White noise treatment spent more time together with the whole group in the same section than Control pigs, with pigs in the Pig treatment being intermediate. There was no difference in the time spent in any of the four sections between the sound treatments or between transported and the stationary treatment.

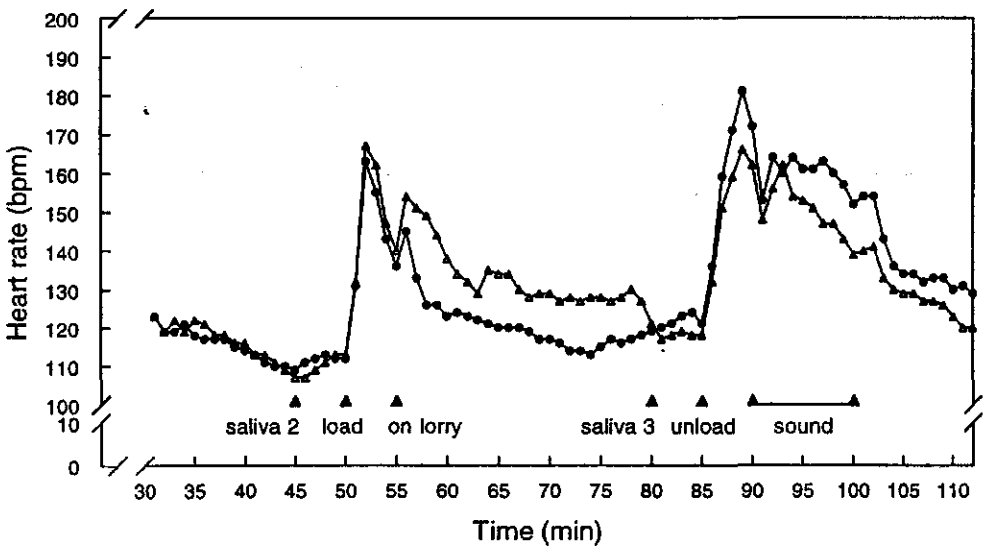

Fig. 1. Mean heart rate for groups of pigs that were transported (Δ) or kept in the stationary lorry (●).

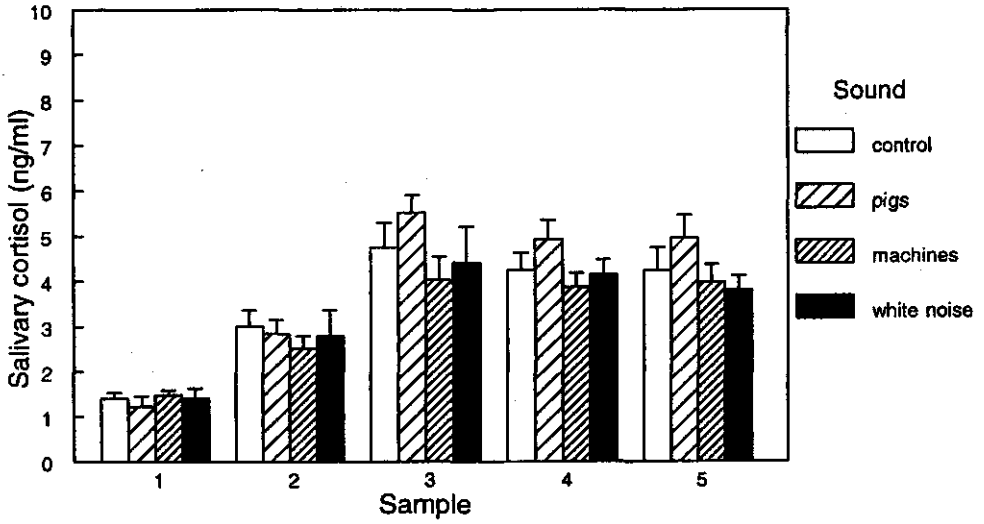

Fig. 2a. Salivary cortisol concentrations (mean ± SEM) for groups of pigs that were kept in the stationary lorry. Sample 1: at the start in the home pen, 2: before loading, 3: after the stationary period, 4: 5 min after the end of the sound exposure period, 5: 10 min after the end of the sound exposure period. Within each treatment, cortisol levels in sample 2 were higher than in sample 1 ($P < 0.001$), and cortisol levels in sample 3 were higher than in sample 2 ($P < 0.001$).

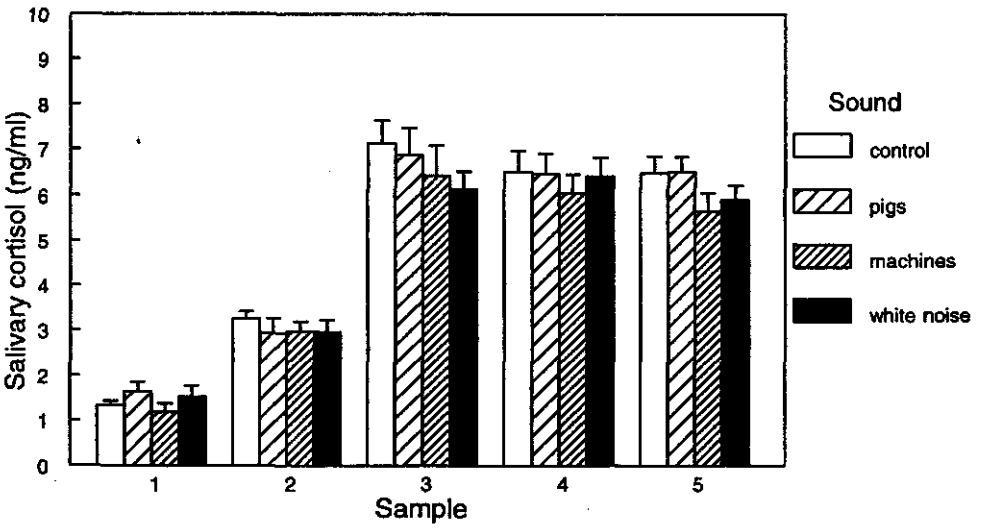

Fig. 2b. Salivary cortisol concentrations (mean ± SEM) for groups of pigs that were transported. Sample 1: at the start in the home pen, 2: before loading, 3: after transport, 4: 5 min after the end of the sound exposure period, 5: 10 min after the end of the sound exposure period. Within each treatment, cortisol levels in sample 2 were higher than in 1 ($P < 0.001$), and cortisol levels in sample 3 were higher than in sample 2 ($P < 0.001$).

Table 2. Mean values and sem's for behavioural parameters in each of the transport x sound treatments

	Sound treatment								Treatment effects	
	Control		Pigs		Machines		White noise		Transport	Sound
	Stationary	Transport	Stationary	Transport	Stationary	Transport	Stationary	Transport	P <	P <
Ambulation (nr. sections)	29.8 ± 2.3^{ab}	25.7 ± 2.4^{cd}	31.9 ± 2.3^{ab}	27.8 ± 2.1^{cd}	26.8 ± 2.7^{bc}	22.7 ± 2.5^{d}	33.3 ± 2.5^{a}	29.2 ± 2.4^{bc}	0.05	ns
Lie (% time)	24.3 ± 3.9	30.2 ± 4.0	23.3 ± 3.9	29.3 ± 3.7	23.2 ± 4.4	29.2 ± 4.3	18.0 ± 4.3	23.9 ± 4.2	0.1	ns
Explore (% time)	48.5 ± 3.7^{a}	40.6 ± 2.9^{b,c}	51.4 ± 2.7^{a}	43.6 ± 3.5^{b}	51.6 ± 3.2^{a}	43.7 ± 3.1^{b}	50.3 ± 3.1^{a}	42.5 ± 4.0^{b}	0.001	ns
All pigs in same section (% time)	15.2 ± 4.4^{a}	12.4 ± 4.2^{a}	22.8 ± 2.8^{ab}	19.9 ± 3.9^{ab}	28.4 ± 4.7^{b}	25.6 ± 4.6^{b}	34.2 ± 5.4^{b}	31.3 ± 4.5^{b}	ns	0.01
In front section (% time)	33.6 ± 4.8	35.2 ± 4.7	35.1 ± 4.7	34.7 ± 4.0	36.2 ± 5.0	38.2 ± 6.8	37.0 ± 4.7	39.9 ± 6.4	ns	ns
In hind section (% time)	23.0 ± 3.1^{ab}	19.0 ± 3.3^{a}	28.8 ± 2.8^{b}	24.8 ± 4.2^{ab}	21.3 ± 3.7^{a}	23.1 ± 3.4^{ab}	23.2 ± 3.4^{ab}	19.2 ± 4.3^{a}	ns	ns

^{a,b} Means within a row lacking a common superscript differ significantly ($p < 0.05$)

Discussion

The results of this study demonstrate that transport had a significant effect on heart rate, cortisol concentrations and ambulation. Although the pigs that were exposed to sound after being handled did not show a response in heart rate or cortisol concentrations, they spent more time close to their group-mates.

The highest peaks in heart rate occurred during loading and unloading, which is in agreement with findings by others (Augustini et al., 1982). Major contributing factors to these peaks are most likely climbing and descending the ramp (van Putten and Elshof, 1978) and being handled (Villé et al., 1993). An increased heart rate during transport compared to previous resting levels has been described by various authors (Augustini et al., 1982; Stephens and Rader, 1982; Villé et al., 1993; Perremans et al., 1996). In addition, this study shows that it is the actual transportation process itself that causes an elevated heart rate, because pigs that were transported had a significantly higher heart rate than pigs in the stationary lorry.

Cortisol release was initially stimulated by strapping the heart rate meters on. The pigs were used to an experimenter in their pen taking saliva samples, but it was the first time they were fitted with heart rate meters. However, the relative contribution of subsequent events to cortisol concentrations was markedly higher. Both the stationary and the transport treatment had a substantial effect on cortisol release. The elevated cortisol concentrations in the stationary group can be attributed to the loading procedure and the novel environment of the lorry. Additional transportation resulted in significantly higher cortisol concentrations. Several other authors have reported a stimulatory effect on cortisol release of short journeys (Becker et al., 1985; Nyberg et al., 1988; Zanella and Unshelm, 1994). Bradshaw et al. (1996) took blood samples regularly after loading pigs and showed that concentrations of total cortisol declined after an initial peak following loading when the vehicle remained stationary. On the contrary, pigs that were transported for 8 h after loading did not show a decline in cortisol for the first 5 h. In agreement with these authors we conclude on the basis of the heart rate and cortisol data that pigs remain stressed following loading and that this response is directly attributable to the transportation process.

During the sound treatment after unloading, both ambulation and heart rate were higher in stationary groups than in transported groups. In addition, the pigs from the stationary treatment also displayed more exploratory behaviours and spent less time lying. Because heart rate is thought to increase with locomotion in mammals (Baudinette, 1978; Smith et al., 1993), physical activity is likely to be the major cause of the increased heart

rate under these circumstances. Furthermore, the transported pigs might have had a higher level of parasympathetic input to the heart during the post-transport period because of the much higher preceding sympathetic activation experienced in the lorry. It is generally thought that the vagal input to the heart tends to become more pronounced as the level of sympathetic activity increases (Stramba-Badiale et al., 1991; Sgoifo et al., 1994).

There were no significant differences in heart rate, cortisol concentrations or activity between sound treatments. Both heart rate and cortisol concentrations were already high before the sounds were presented, due to the transport-associated handling. It may be that pigs do respond with a change in heart rate and cortisol when they are not handled beforehand. However, there was an interesting behavioural difference. Pigs exposed to the sound of Machines or White noise spent more time together with their group-mates in the same section than pigs in the Control treatment, with the pigs exposed to the Pig sound being intermediate. Social contact is an important aspect for pigs (Barnett et al., 1981; Ladewig and Matthews, 1988; Fraser and Broom, 1990; Harri, 1992), and being in a group may provide some protection from the effects of stressors, as has been reported in other species (Gunnar et al., 1980; Taylor, 1981; Chen and Wills, 1985). We tentatively suggest that the sounds implied a threat to the pigs to some extent. However, this threat is not reflected in elevated cortisol concentrations and heart rate, indicating that pigs may cope with the sound stimuli by staying close together with their conspecifics. To test this hypothesis, the responses of individual pigs exposed to sound should be examined.

The question remains whether the pigs attributed meaning to the sounds used and recognize the biological content of the pig sound, because they behaviourally responded to a greater extent to the sound of machines and white noise than to the sound of pigs. Others have reported opposite results after testing 1-month old piglets in a familiar room, in the presence of a companion pig (Talling et al., 1996). The piglets responded to farm and transport recordings with a reduction in ambulation score, which the authors attributed to the recognition of vocalisations of other piglets in those recordings. The absence of response to an abattoir recording was attributed to the aural complexity of the recording, but also the farm and transport recordings contained other sounds like a ventilation fan and engine sounds. Therefore, it remains unclear which characteristics of a recording a pig responds to.

Hearing of 4-month old pigs ranges from 42 Hz to 40,5 kHz with a region of best sensitivity from 250 Hz to 16 kHz (Heffner and Heffner, 1990). As the equipment used

in this experiment did not replay above 16 kHz, it may be that the pigs missed part of the aural information. However, pigs do not use ultrasonic sounds for communication. Sub-adult squeals have a fundamental tone at about 600 Hz and an overtone at 4 kHz (Kiley, 1972), and these tones were thus reproduced by the equipment. A possibility is that the pigs had been habituated to the sound of squeals during the fattening period, and learned that squeals were not associated with an immediate threat. The unfamiliarity of the sound of machines and white noise could be reasons that these sounds were perceived as a threat.

In conclusion, pigs stay close to their group-mates when exposed to lairage sounds but heart rate or cortisol concentrations are not affected. The impact of transportation on the physiology and behaviour is considerable.

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Chapter 6

Effect of regular moving and handling on behavioural and physiological responses of pigs to preslaughter treatment and consequences for subsequent meat quality

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Submitted

Abstract

The effects of regular moving and handling during the fattening period on behavioural and physiological responses of pigs during preslaughter treatment, and consequences for meat quality were studied. From the age of 10 wk onwards, 144 pigs were housed in groups of four (2 gilts and 2 castrates) and subjected to one of the following treatments. The Environment treatment allowed pigs to move freely for 8 min outside their home pen. Then the pigs were transported in a box for 2 min, after which they were returned to their home pen. In the Handling treatment, an experimenter remained for 3 min in the pen, and whenever a pig made contact, it was gently stroked. The experimenter then walked for a further 1 min, without attempting to pat or stroke any pigs but subsequently held each pig in a tight grip for about 5 s. This entire procedure was then repeated. A Control treatment was also included whereby the pigs were subjected to no treatment. The Environment and Handling treatment were applied twice a week at the age of 15, 17, 19, 21 and 23 wk. At 25 weeks of age, pigs were transported to the abattoir. They were held unmixed in the lorry and in lairage, and were manually stunned. The stockmen needed significantly less time to move Environment pigs out of their pen and into the transport box. There were no differences between treatments in salivary cortisol concentrations before or after transport. Environment and Handling pigs had paler meat than Control pigs. Glycogen content at 1 h post-mortem and waterholding capacity were lower in Environment pigs compared to Control, but this did not lead to a higher incidence of PSE-meat. It is concluded that those pigs that had experience with leaving their home pen were much easier to handle at loading. Pigs that are easier to move are less likely to be subject to rough handling, which implies improved welfare, while the workload for stockmen is reduced. Differences in meat quality due to treatment were slight.

Introduction

Preslaughter treatment involves a number of aspects such as moving the pigs out of the pen, loading, transport, unloading in lairage and moving to the stunning pen. All these aspects may influence behaviour, physiology and resultant meat quality (Tarrant, 1989; Troeger, 1989; Stephens and Perry, 1990). It has been suggested that the extent to which pigs can cope with the stress associated with preslaughter treatment may be influenced by rearing conditions (Barton-Gade and Blaabjerg, 1989; Grandin, 1989; Hunter et al., 1997).

During the fattening period, pigs are usually kept in the same pen under

intensive housing conditions. This is often attended with little environmental variation. It has been suggested that pigs kept in a barren environment show a high degree of reactivity to novel stimuli and in some cases may be very disturbed by them (Stolba and Wood-Gush, 1980). Another aspect of routine husbandry is that the only contact pigs have with humans is usually unpleasant, e.g. cutting teeth, tail docking, castration for the males and vaccinations. Several authors report that additional pleasant handling will make pigs less fearful to humans (Hemsworth et al., 1994a; Tanida et al., 1994). However, there is only limited evidence as to how regular handling or environmental variation may affect responses during preslaughter treatment.

Therefore, the following experiment was designed to examine the effects of 1) regular exposure of pigs to another environment than their home pen and 2) regular contact with humans, on behavioural and physiological responses to preslaughter treatment, and consequences for subsequent meat quality.

Material and Methods

Animals and housing

Subjects were 144 pigs (Great Yorkshire x (Great Yorkshire x Dutch Landrace)), 72 gilts and 72 castrates. At the age of 10 weeks, the pigs (average weight 28 kg) were housed in 36 groups of four pigs, each group consisting of two gilts and two castrates. Groups were housed in pens with fully slatted concrete floors (1.8 x 1.6 m) in a room that was enclosed and temperature-controlled. The pigs were provided with ad libitum food and water throughout the study. Main artificial lights were on from 0600 until 1800.

Treatments

Groups were assigned to one of three treatments: the Environment treatment (n = 12), the Handling treatment (n = 12) and a Control treatment (n = 12).

1) *Environment treatment*. The door of the pen was opened, allowing the pigs to voluntarily enter the passageway. The passageway was blocked on two sides by boards, creating an area of 7 m x 1 m. The pigs were allowed to move freely for 8 min. Then they were gently moved by two handlers with boards into a transport box on wheels situated at the end of the passageway, just outside the room. Next, the box was driven through another part of the building for 2 min. Then the transport box was returned to the passageway. The pigs were unloaded and driven with boards to their pen. During the whole procedure, the handlers did not enter the area in which the pigs

were, i.e. pigs and humans were always separated by a board.

2) *Handling treatment*. The experimenter entered the pen, and moved to one of the front corners. When the pigs were 15 wk old, the experimenter adopted a stationary squat posture for 3 min. As soon as a pig initiated contact (sniffing, chewing or biting overalls or boots), it was gently stroked on its nose and behind its ears. After 3 min, the experimenter walked through the pen for one min without attempting to pat or stroke any pigs but subsequently held each pig in a tight grip (i.e. with both hands holding the neck) for about 5 s. This combination of handling treatments was chosen to accustom the pigs to both pleasant and potentially unpleasant but not harmful handling. Then the experimenter moved to the opposite back corner and the whole procedure was repeated. When the pigs were 17 wk of age and above, the procedure was slightly different due to the reduced fear and increased size of the animals. The experimenter adopted an erect posture for 1 min in one of the front corners of the pen, followed by slowly walking through the pen for 2 min. During these 3 min, the pigs that initiated contact were gently stroked. Next, the experimenter walked through the pen for 1 min without attempting to pat or stroke any pigs but subsequently held each pig in a tight grip for 5 s. Then the experimenter moved to the opposite back corner and the whole procedure was repeated.

3) *Control treatment*. No treatment.

The Environment and Handling treatment were applied during 10 sessions, i.e. twice a week at the age of 15, 17, 19, 21 and 23 wk. The sessions took place between 0900 and 1200. During the course of the experiment, two pigs in the Environment treatment, two pigs in the Handling treatment and three pigs in the Control treatment were removed due to various health problems. These pigs all originated from different groups.

Transport and lairage procedure

At the age of 25 wk, the pigs were delivered to a commercial slaughterhouse. Six groups were transported per day, i.e. two groups of the Environment treatment, two groups of the Handling treatment, and two groups of the Control treatment. The procedure for each group was as follows. Feed was withdrawn approximately 20 h before slaughter. At 0900, the pigs were moved out of the pen with a board by two trained stockmen, and moved into the transport box situated at the end of the passageway (average distance 8 m). The pigs were transported in the transport box to the lorry and loaded. This procedure was repeated for the remaining five groups. The

stockmen were not informed about the treatment category of the groups. Treatment groups were loaded alternately. The groups were penned separately on the same deck of the lorry (0.6 m^2/pig). The average duration of transport to the slaughterhouse was 45 min. After a wait of 45 min in the stationary lorry, each group was separately unloaded and kept unmixed in lairage for 5 min. At 1200, the first group was driven to the stunning pen and the pigs were slaughtered according to normal commercial practices after manual electrical stunning.

Behaviour

Behavioural parameters collected during loading at the farm were 1) latency time for each pig to leave the pen, 2) latency time to enter the transport box. Behaviour in lairage was recorded on videotape. Behavioural variables were scored from video tape with a behavioural software package (The Observer 3.0, Noldus Information Technology, Wageningen, The Netherlands). The holding pen in lairage was divided in four imaginary sections of 2 x 2 m, and ambulation score of each pig was measured by counting how many sections the pig entered. Duration of exploration (sniffing, biting, rooting, chewing) of walls and floor was also scored.

Saliva collection and cortisol analysis

Cortisol in saliva is essentially in the free form, providing a good indication of levels of free biologically active cortisol circulating in blood plasma (Kirschbaum and Hellhammer, 1989; Parrott and Misson, 1989; Parrott et al., 1989). Saliva samples were collected 1) in the home pen before loading and 2) immediately upon arrival at the slaughterhouse, in the lorry. Saliva samples were collected by allowing the pig to chew on two cotton buds until they were thoroughly moistened. It took on average 1 min to take a saliva sample. The cotton buds were stored in test tubes, kept on ice and subsequently centrifuged at 5000 *g* for 5 min to remove the saliva which was stored at -20°C until assay. Cortisol concentration was measured using a commercial RIA kit (Coat-a-Count, Diagnostic Products Corporation, Apeldoorn, The Netherlands) modified for pig salivary cortisol (Ruis et al., 1997).

Muscle samples

A biopsy needle was used to obtain muscle samples (± 50 mg) from the longissimus muscle (LM) 5 cm caudal from the last rib at 60 min post-mortem for assessment of glycogen concentration. The muscle samples were frozen in liquid

nitrogen within 10 s of sampling and stored at -80°C until analysis. Samples were freeze-dried overnight. Freeze-dried muscle samples were dissected free from connective tissue, blood and fat, and powdered with a pulverizing machine (type MM2, Retsch B.V., Ochten, The Netherlands). Five to 10 mg of the powdered muscle tissue was suspended in 200 μl of 0.5 M perchloric acid for the measurement of glycogen. Conversion of glycogen to glucose by amyloglucosidase was conducted (method according to Haagsma et al., 1981). Glucose was extracted by suspension of powdered material in 0.5 M perchloric acid (20 to 40 mg dried tissue/mL of acid). The suspension was centrifuged (10 min, 1500 *g*) and 500 μl clear supernatant neutralized (pH 6.5 to 7.0) with 35 μl 5.4 M KOH. Glycogen and glucose solutions were centrifuged (10 min, 1500 *g*) and 200 μl clear supernatant was diluted 1:1.25 in 2.0 M tri-ethanolamine buffer. The total amounts of glucose and glucose-6-phosphate (G-6-P) were measured using a commercial kit (Glucose Kit No. 115, Sigma Diagnostics, Zwijndrecht, The Netherlands). Glycogen content was determined after a correction for free glucose and G-6-P already present in the extract.

Meat quality measurements

Hot carcass weights were recorded. Temperature and pH of the LM and the semimembranosus muscle (SM) were measured at 45 min post-mortem. Muscle pH was measured with a pH-meter connected to an Ingold electrode (Xerolyte, type LOT 406). Rigor mortis in the SM was determined with a rigormeter at 45 min post-mortem (Sybesma, 1966).

An LM sample was taken at the 3th-4th lumbar vertebra and its waterholding capacity was measured at 26 h post-mortem according to the filter paper absorption method (Kauffman et al., 1986). After a 30-min bloom period the colour was determined in triplicate by measuring L*-, a*-, and b*-values with a Hunter Labscan (D65, 10° standard observer). The percentage drip loss was measured on a LM sample that was packaged and stored at 0-2°C for 42 h. PSE meat was defined according to the criteria of Kauffman et al. (1993) with Hunter L^* values > 58 and a drip loss > 5%.

Statistical analysis

Group means were analysed with an analysis of variance model. Factors in the analysis were 'Slaughter day' (1 to 6) and 'Treatment' (Environment, Handling and Control). A paired t-test (Conover, 1980) was used to analyse increase in salivary

cortisol concentrations. Differences in incidence of PSE were analysed using a chi-square test (Conover, 1980). All calculations were performed with the statistical programming language Genstat 5 (1993).

Results

During the course of the Environment treatment sessions, pigs showed a reduction in latency time to leave the pen voluntarily (Fig. 1). On the day of slaughter stockmen needed less time to move Environment pigs out of their pen compared to Handling and Control pigs (Table 1). They also needed less time to move the pigs into the transport box. It was observed (not measured) that Environment pigs showed an inclination towards leaving the pen voluntarily and walking straight to the transport box. Transport increased salivary cortisol in all treatments ($P < .01$). Cortisol before or after transport, and increase in salivary cortisol did not differ between the three treatments. In lairage, exploration and ambulation did not differ between treatments.

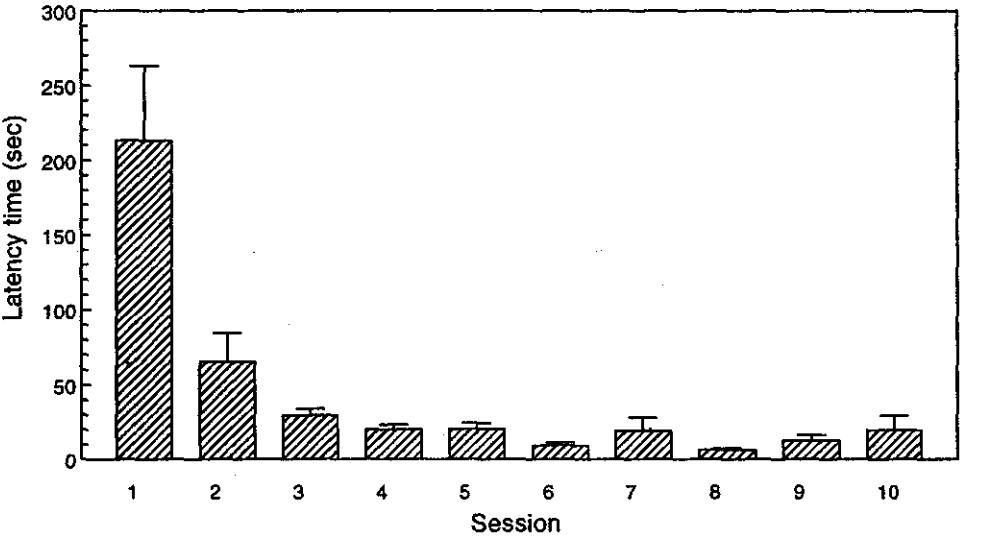

Fig. 1. Mean latency time (± sem) to leave the pen voluntarily during the 10 sessions of the Environment treatment (n=12).

Table 1. Mean values and sem's of behavioural and physiological parameters from pigs which were subjected to the Environment, Handling or Control treatment during the fattening period

	Treatment					
	Environment (n=12)		Handling (n=12)		Control (n=12)	
	Mean	SEM	Mean	SEM	Mean	SEM
Latency time to leave pen (sec)	26.7^{x}	2.0	50.1^{y}	3.3	54.1^{y}	4.4
Latency time to enter transport box (sec)	72.7^{x}	6.0	144.1^{y}	13.2	169.6^{y}	13.2
Exploration in lairage (% time)	35.5	6.0	38.9	3.1	40.0	3.7
Ambulation in lairage (nr sections entered)	19.2	2.5	20.4	3.2	17.3	3.2
Salivary cortisol before transport (ng/ml)	2.13	.34	2.20	.21	2.48	.22
Salivary cortisol after transport (ng/ml)	8.24	.71	8.96	.64	8.26	.69
Increase in salivary cortisol (ng/ml)	6.10	.82	6.77	.51	5.74	.74

^{x,y} Means within a row lacking a common superscript letter differ ($P < .05$)

Meat quality characteristics are shown in Table 2. Environment pigs showed a higher temperature in LM at 45 min after slaughter than Control pigs. At 45 min post mortem pH in SM was significantly lower in Environment pigs than in Handling pigs. Glycogen content one hr after slaughter and waterholding capacity were lower in Environment pigs than in Control pigs. Meat colour was lighter for the Environment and Handling pigs than for the Control pigs. In all treatments, meat showed a relatively pale colour. There were no differences in incidence of PSE-meat (Environment: 9 pigs, Handling: 7 pigs, Control: 7 pigs; ns.).

Table 2. Mean values and sem's of meat quality characteristics from pigs which were subjected to the Environment, Handling or Control treatment during the fattening period

	Treatment					
	Environment (n=12)		Handling (n=12)		Control (n=12)	
	Mean	SEM	Mean	SEM	Mean	SEM
Carcass wt (kg)	86.3	1.3	85.3	1.6	87.3	1.9
T45 min SM (°C)	40.31	.12	40.33	.11	40.28	.09
T45 min LM (°C)	40.42^{x}	.15	40.15^{xy}	.11	40.03^{y}	.13
pH45 min SM	6.22^{x}	.05	6.40^{y}	.06	6.35^{xy}	.04
pH45 min LM	6.31	.04	6.40	.05	6.39	.03
Rigor mortis SM	7.5	.7	7.1	.6	7.3	.4
Glycogen (μmol/g wet muscle)	29.15^{x}	2.04	34.83^{xy}	1.98	37.81^{y}	1.64
Fluid wt of filter paper (mg)	49.6^{x}	.6	42.6^{xy}	4.9	36.7^{y}	2.1
Drip loss in %	4.4	.3	4.3	.4	3.9	.3
Hunter L*-value	59.03^{x}	.52	58.22^{x}	.67	57.37^{y}	.35
Hunter a*-value	6.96	.13	6.76	.16	6.71	.13
Hunter b*-value	14.84^{x}	.12	14.62^{xy}	.15	14.27^{y}	.10

^{x,y} Means within a row lacking a common superscript letter differ ($P < .05$)

Discussion

The present results show that the time needed to load pigs, that had been repeatedly let out of their pen during the fattening period, was significantly reduced. A beneficial consequence was that the workload for the stockmen was reduced. The experience of having been outside the home pen during the fattening period thus

seems to be an important factor in increasing the willingness to move at preslaughter handling. This is supported by Abbott et al. (1994), who moved pigs once a week for 3 weeks consecutively and found that the animals left their pens more quickly at preslaughter loading. However, it is not clear to which extent the pigs had contact with handlers during moving in that study. Our Handling pigs were not easier to move than the Control pigs, despite their experience with contact with humans. The lack of experience with going outside the home pen thus seems to be a decisive factor. Other reasons could be that the pigs were loaded by other persons than the experimenters. Research by Tanida and Nagano (1996) suggests that pigs are able to distinguish between people. However, Hemsworth et al. (1994b) showed that the behavioural response of commercial pigs to one handler is likely to extend to other humans. Another explanation may be that frequent handling may result in the pigs becoming too tame which makes driving difficult, as suggested by Grandin (1989).

Despite the behavioural differences indicating that Environment pigs showed less resistance to the loading procedure there were no differences in basal cortisol levels or cortisol levels after transport. Transport is a considerable stressor (e.g. Stephens and Perry, 1990; Lambooy and van Putten, 1993; Warriss and Brown, 1994) and may eventually have resulted in a maximum response of the adrenal cortex, while cortisol levels immediately after loading may have been different.

Significant differences were present for several meat quality parameters. Temperature in LM at 45 min post-mortem was increased and glycogen levels at 1 h post-mortem and waterholding capacity were decreased in the Environment treatment. Pigs in the Environment and Handling treatments showed paler meat than Control pigs. These results suggest an enhanced post-mortem glycogen breakdown in Environment pigs although this did not lead to a significantly higher incidence of PSE meat. Also the pigs in the other treatments showed a tendency in the direction of PSE, considering the high light reflection values. It has been observed by other authors that a lower incidence of DFD but a higher incidence of properties of PSE occurs in pigs of certain breeds (Monin and Sellier, 1985), and in pigs that have been exposed to moderate exercise (Essén-Gustavsson et al., 1988; Enfält et al., 1993) or preslaughter treatment that minimizes stress (Barton-Gade and Blaabjerg, 1989). These authors suggest that the accelerated post-mortem breakdown of glycogen leading to PSE-properties may be caused by high glycogen levels before slaughter. The Environment treatment not only accustomed the pigs to being outside their home pen, but also provided the pigs with moderate exercise each time. Environment pigs were faster to

load on the lorry at the day of slaughter which was most likely associated with less physical activity. These factors may have contributed to higher glycogen levels preslaughter. Furthermore, for all treatments handling in lairage was less stressful than under normal commercial circumstances: pigs were not mixed and they were moved over a short distance within their group. These circumstances may eventually have caused the pale colour observed in all treatments.

Implications

The results show that pigs that were regularly exposed to another environment than their home pen, were easier to handle when transported to the slaughterhouse than control pigs or pigs that were regularly handled. Pigs that are easier to move are less likely to be subject to rough handling, which implies improved welfare, while the workload for stockmen is reduced. Meat was slightly paler than normal for all treatments, probably because of the careful handling procedure in lairage and absence of mixing. A possible relationship between preslaughter handling, high energy reserves at slaughter and post-mortem accelerated breakdown of glycogen merits further research.

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Chapter 7

Effect of housing system on responses of pigs to preslaughter treatment and consequences for meat quality

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Abstract

The effects of moderately enriched housing conditions on behavioural and cortisol responses during preslaughter treatment were studied in 48 slaughter pigs, as well as the consequences for meat quality. Pigs were either raised in intensive housing conditions ("Poor" treatment: standard farrowing crates followed by standard rearing and fattening pens) or in extensive conditions ("Enriched" treatment: larger farrowing pens followed by larger rearing and fattening pens, all provided with straw). During preslaughter treatment, stockmen needed significantly less time to load pigs from Poor housing conditions. These pigs may have experienced a lack of stimulation in the barren conditions of their home pen, while having experience on three occasions during the fattening period with being outside the pen. Consequently, they may have felt more motivated to leave their pens in order to explore a new environment than pigs from the Enriched housing pens. Enriched pigs showed higher salivary cortisol levels before transport, which may be due to the omission of straw after cleaning of the pens that day. The cortisol increase in response to transport was higher in Poor pigs, suggesting that either transport induced a larger cortisol release in Poor pigs, or that a maximum stimulation of the adrenal cortex in Enriched pigs was reached. Enriched pigs showed more exploratory behaviours in lairage. Glycogen content 1 h post-mortem showed a trend to be lower in pigs from Enriched pens, while levels did not differ at sticking or on the previous day. This suggests an enhanced post-mortem glycogen breakdown in Enriched pigs, but this did not result in differences in any of the meat quality variables measured.

Introduction

Increased public interest in meat from more welfare-friendly systems has led to an increased number of farms that rear pigs in extensive housing conditions (Kleijn et al., 1991; PVE, 1997). It has been shown that these systems allow a broad range of behaviour patterns. Pigs reared in straw pens show more exploratory behaviours and less manipulation (nosing, chewing, tailbiting) of penmates (Schouten, 1991; Beattie, 1995a; Lyons, 1995; Petersen, 1995; De Jong et al., 1998), less aggressive behaviour and less time spent inactive (Beattie, 1995a; Lyons 1995). These studies indicate that welfare is improved by enrichment. Furthermore, it is suggested by some authors that pigs kept in a barren environment, as opposed to pigs kept in enriched housing conditions, show a high degree of reactivity to novel stimuli and in some cases may be very disturbed by them (Stolba and Wood-Gush, 1980).

Since preslaughter treatment is always associated with novel stimuli like handling, loading and unloading, transport, and being kept in lairage, it may be that pigs from extensive housing conditions can cope better and react less adversely to preslaughter treatment than pigs from intensive housing systems. Some authors have indeed observed calmer behaviour of extensively reared pigs during preslaughter handling (Warris et al., 1983; Barton-Gade and Blaabjerg, 1989), but did not quantify these responses.

The extent to which pigs can cope with the factors associated with preslaughter treatment is not only relevant to the welfare of the animals but may also be a decisive factor in ultimate meat quality, as psychological and physical stress immediately before slaughter is thought to lead to impaired meat quality (Tarrant, 1989). Results on meat quality from intensively vs. extensively reared pigs have been reported (Warris et al., 1983; van der Wal et al., 1993; Barton-Gade, 1995; Enfält et al., 1997), but do not agree. Furthermore, these studies concerned pigs that were reared outdoors. However, little regard has been paid so far to the effects of moderate enrichment on responses of pigs to preslaughter treatment and consequences for meat quality. Moderate enrichment, i.e. indoor rearing with extra space allowance and the additional supply of straw, is easier to put into practice.

The present experiment was designed to investigate the effects of moderately enriched housing conditions, i.e. indoor pens with extra space and supply of straw, on behavioural and cortisol responses of pigs during preslaughter treatment, and the consequences for meat quality.

Materials and Methods

Subjects and experimental design

Subjects were 48 crossbred slaughter pigs (Great Yorkshire x (Great Yorkshire x Dutch Landrace) studied by De Jong et al. (1998). Two treatments (Enriched or Poor housing) were applied to three successive replicates of 16 pigs. Within a replicate, two groups of four pigs were assigned to each treatment. Each group consisted of two castrated males and two gilts originating from the same litter.

Rearing and fattening environments

Enriched pigs were farrowed and nursed in farrowing pens (7.2 m^2) with a concrete lying area covered with straw (2.4 x 1.75 m) and a concrete slatted area (2.4 x 1.25 m). At weaning (4 weeks of age) three castrates and three gilts per litter were selected and the sow and not-selected piglets were removed. At 10 weeks of age two

castrates and two gilts per group were eventually selected and relocated to enriched fattening pens measuring 2.9 x 1.6 m, with half concrete area covered with straw and half concrete slats.

Poor pigs were farrowed and nursed in standard farrowing crates (4.2 m^2, one-third concrete area, two-third metal slats) with the sow restrained between bars. At weaning (4 weeks of age) three castrates and three gilts per litter were selected and moved to fully slatted pens measuring 2.4 x 1.25 m. These pens were in the same room as the enriched farrowing pens. At 10 weeks of age two castrates and two gilts per group were eventually selected and relocated to standard fattening pens measuring 2.1 x 1.6 m, with half concrete area and half concrete slatted floor. Enriched and Poor fattening pens were in the same room.

Throughout the experiment, animals were given ad libitum access to food and water. Environmental temperature was kept between 21-23 °C. Artificial lights were on from 6.00 - 18.00 h. All pens were cleaned daily at 6.00 h whereby fresh straw was provided in the enriched pens.

Transport and lairage procedure

Full details of home pen behaviour, physiology, and behavioural and physiological responses to several tests are given in De Jong et al. (1998). For each of the three replicates, the pigs were delivered to a commercial slaughterhouse at the age of 26 weeks. Feed was withdrawn approximately 20 h before slaughter. At 09.45 h, the pigs were moved out of the pen with a board by two trained stockmen, and moved into the transport box situated at the end of the passageway (average distance 10 m). The pigs were transported in the transport box to the lorry and loaded. Enriched and Poor groups were loaded alternately. The four groups were penned separately on the same deck of the lorry (0.6 m^2/pig). The average duration of transport to the slaughterhouse was 40 min. After a wait of 45 min on the stationary lorry, each group was separately unloaded and kept unmixed in lairage for 5 min. At approximately 12.00 h, the first group was driven to the stunning pen and the pigs were manually stunned.

Behaviour

Behavioural parameters collected during loading at the farm were 1) time needed to move each pig out of the pen, 2) time needed to move each pig into the transport box.

Inside the lorry, two cameras were mounted at the ceiling, each camera

encompassing two pens. Continuous recordings were made during transport and during the waiting period at the slaughterhouse. In lairage, behaviour was continuously recorded with a camcorder. Behaviours were scored from video tape with the behavioural software program The Observer 3.0 (Noldus Information Technology, Wageningen, The Netherlands) for the following periods: 1) first 15 min period of transport, 2) second 15 min period of transport, 3) 5 min period on stationary lorry after arrival at slaughterhouse with the experimenter taking saliva samples (see Salivary cortisol section), 4) 30 min period on stationary lorry following saliva sampling and 5) 5 min period in lairage. Percentage of time spent standing, sitting and lying was scored. For the lorry recordings, each pen in the lorry was divided in two imaginary sections of 2.20 x 1.08 m, and ambulation of each pig was measured by counting how many times the pig entered another section. For scoring of ambulation in lairage, the holding pen in lairage was divided in four imaginary sections of 2 x 2 m. Percentage of time spent exploring (sniffing, biting, rooting, chewing) walls and floor in the lorry and in lairage was scored. In addition, percentage of time spent initiating contact with the experimenter during saliva sampling (i.e. sniffing, chewing or bitting overalls or boots) was scored.

Salivary cortisol

Cortisol in saliva is essentially in the free form, providing a good indication of levels of free biologically active cortisol circulating in blood plasma (Kirschbaum and Hellhammer, 1989; Parrott and Misson, 1989; Parrott et al., 1989). Saliva samples for assessment of cortisol were collected in the home pen immediately before loading, and in the lorry immediately upon arrival at the slaughterhouse.

Saliva samples were collected by allowing the pig to chew on two cotton buds until they were thoroughly moistened. It took on average 1 min to take a saliva sample. The cotton buds were stored in test tubes, kept on ice and subsequently centrifuged at 5000 *g* for 5 minutes to remove the saliva which was stored at -20°C until assay. Cortisol concentration was measured using a commercial RIA kit (Coat-a-Count, Diagnostic Products Corporation, Apeldoorn, The Netherlands) modified for pig salivary cortisol (Ruis et al., 1997).

Plasma cortisol

Blood samples were collected at exsanguination for assessment of cortisol. Blood was collected in heparinized tubes, kept on ice and centrifuged (10 min, 4000 *g*). Plasma was stored at -20°C until assay. Total cortisol concentration (bound + free cortisol) was measured using a commercial RIA kit (Coat-a-Count, Diagnostic Products Corporation, Apeldoorn, The Netherlands). Determination by an eight-fold measurement of a sample with a mean cortisol concentration of 158.75 ng/ml resulted in an intra-assay coefficient of 6.2%. Addition of 498.37 ng cortisol per ml plasma resulted in a recovery of 118%. Parallelity of the calibration curve with a plasma dilution curve (dilutions 1:2, 1:4, 1:8 and 1:16) was tested for a sample of 362.45 ng/ml and 289.96 ng/ml. In percentage of the expected concentrations, the mean measured value was 117%. The minimal detectable dose or sensitivity (concentration at 95% of the maximum binding) of the assay was approximately 3.16 ng cortisol per ml.

Muscle samples

From all subjects, muscle samples from the dorsal muscle or longissimus muscle (LM) were taken for assessment of glycogen concentration 1) one day before slaughter, 2) immediately after sticking, and 3) 60 min post-mortem. The muscle samples were taken with a shot biopsy device (as described by Geverink et al., subm.a). The biopsy shot was applied 5 cm caudal from the last rib, around 5 cm from the dorsal line. The muscle samples (± 50 mg) were frozen in liquid nitrogen within 10 sec of sampling and stored at -80°C until analysis. Samples were freeze-dried overnight. Freeze-dried muscle samples were dissected free from connective tissue, blood and fat, and powdered with a pulverizing machine (type MM2, Retsch B.V., Ochten, Netherlands). Five to 10 mg of the powdered muscle tissue was suspended in 200 µl of 0.5 M perchloric acid for the measurement of glycogen. Conversion of glycogen to glucose by amyloglucosidase was conducted (method according to Haagsma et al., 1981). Glucose was extracted by suspension of powdered material in 0.5 M perchloric acid (20 to 40 mg dried tissue/mL of acid). The suspension was centrifuged (10 min, 1500 g) and 500 µl clear supernatant neutralized (pH 6.5 to 7.0) with 35 µl 5.4 M KOH. Glycogen and glucose solutions were centrifuged (10 min, 1500 g) and 200 µl clear supernatant was diluted 1:1.25 in 2.0 M tri-ethanolamine buffer. The total amounts of glucose and glucose-6-phosphate (G-6-P) were measured using a commercial kit (Glucose Kit No. 115, Sigma Diagnostics, Zwijndrecht, The Netherlands). Glycogen content was determined after a correction for free glucose and G-6-P already present in the extract.

Meat quality variables

Temperature and pH of the LM and the semimembranosus muscle (SM) were measured at 45 min post-mortem. Muscle pH was measured with a pH-meter connected to an Ingold electrode (Xerolyte, type LOT 406). Rigor mortis in the SM was determined with a rigormeter at 45 min post mortem (Sybesma, 1966).

An LM sample was taken at the 3rd-4th lumbar vertebra. Waterholding capacity was measured at 26 h post-mortem according to the filter paper absorption method (Kauffman et al., 1986). After a 30-min bloom period the colour was determined in triplicate by measuring L*-, a*-, and b*-values with a Hunter Labscan (D65, 10° standard observer). The percentage drip loss was measured on a LM sample that was packaged and stored at 0-2°C for 42 h. PSE meat was defined according to the criteria of Kauffman et al. (1993) with Hunter L^* values > 58 and a drip loss > 5%.

Statistical analysis

A group of animals in a pen was considered the experimental unit and therefore group means were analysed. One castrate had to be removed from the Poor housing treatment in the third replicate, because of health problems. Behavioural data from the transport recordings for the second replicate were omitted due to a failure in the recording equipment. Data were analysed with an analysis of variance model. Factors in the analysis were 'Replicate' (First, Second, Third) and 'Treatment' (Enriched, Poor). All calculations were performed with the statistical programming language Genstat 5 (1993).

Results

Behavioural data are shown in Table 1. The stockmen needed significantly less time to move pigs from Poor housing conditions out of the pen than pigs from Enriched housing conditions. Poor pigs were also faster to load into the transport box. On the lorry, ambulation and exploration did not differ between treatments. During the second half of the transport period, Enriched pigs spent more time standing. In lairage, ambulation did not differ between treatments. However, Enriched pigs spent significantly more time exploring the holding pen.

Cortisol results are presented in Table 2. Enriched pigs show higher salivary cortisol levels before and after transport, but cortisol rise was higher in Poor pigs. Enriched pigs had higher, though not significantly, cortisol levels in sticking blood.

Table 1. Mean values and sem's of several behavioural characteristics of pigs raised in enriched or poor environments

Variable	Housing system				P <
	Enriched		Poor		
	Mean	SEM	Mean	SEM	
Farm					
Out of pen (sec)	27.3	4.3	13.7	1.5	0.001
Into transport box (sec)	93.7	12.5	56.8	11.9	0.01
1st 15 min transport					
% time standing	98.8	0.1	98.6	0.2	ns
ambulation[1]	8.4	1.0	8.3	0.2	ns
% time exploring	10.8	0.5	7.4	3.0	ns
2nd 15 min transport					
% time standing	95.4	3.4	85.1	6.0	0.05
ambulation[1]	7.2	1.3	4.9	1.4	ns
% time exploring	17.5	5.7	13.9	5.3	ns
5 min presence experimenter					
% time standing	85.3	7.9	95.6	2.6	ns
ambulation[1]	2.9	0.7	3.1	2.6	ns
% time exploring	12.2	5.6	5.7	2.6	ns
% time contact	12.6	7.2	18.5	5.8	ns
30 min stationary					
% time standing	56.7	13.9	65.7	9.0	ns
ambulation[1]	8.5	2.6	8.4	1.6	ns
% time exploring	16.1	5.8	17.9	2.4	ns
lairage					
% time standing	100	0	100	0	ns
ambulation[1]	5.4	0.4	5.4	0.8	ns
% time exploring	49.2	4.0	36.9	4.3	0.05

[1]nr. sections crossed in holding pen

Table 2. Mean values and sem's of cortisol levels in saliva and plasma in pigs raised in enriched or poor environments

Variable	Housing system				P <
	Enriched		Poor		
	Mean	SEM	Mean	SEM	
Salivary cortisol before transport (ng/ml)	8.63	1.27	2.60	0.37	0.001
Salivary cortisol after transport (ng/ml)	12.40	0.99	9.28	0.54	0.01
Salivary cortisol rise (ng/ml)	3.63	0.58	6.68	0.80	0.001
Plasma cortisol (ng/ml)	151.5	13.30	131.68	9.10	ns

Glycogen concentrations in LM samples taken one day before slaughter and at sticking did not differ between treatments (Table 3). At 1 h postmortem, the glycogen content tended to be lower for Enriched pigs. None of the measured meat quality variables differed between treatments.

Discussion

This study showed that during preslaughter treatment, Poor pigs were easier to load and displayed lower cortisol levels before and after transport than Enriched pigs, but there were no differences in meat quality.

During loading, it took stockmen more time to move the pigs from the Enriched treatment out of their pen and into the transport box. This is in agreement with work by Beattie et al. (1995b), who found that gilts reared in enriched environments indoors were more difficult to drive in a handling test. However, Warris et al. (1983) and Barton-Gade and Blaabjerg (1989) judged the behaviour of free range pigs reared outdoors as more willingly to move than pigs fattened indoors under moderately intensive conditions. The pigs from both housing systems in this study had been moved out of their pen before, at 14, 17 and 25 weeks of age for several tests (see de Jong et al., 1998). For the pigs from the Poor treatment, which may have experienced a lack of stimulation in the barren conditions of their home pen, these tests may have involved a positive component, i.e. the possibility to explore a new environment, while pigs from

Table 3. Mean values and sem's of several meat quality characteristics from pigs raised in enriched or poor environments

Parameter	Housing system				P <
	Enriched (n=6)		Poor (n=6)		
	Mean	SEM	Mean	SEM	
Live wt (kg)	121.2	3.1	114.6	4.1	ns
glycogen (umol/g wet muscle)					
1 day ante-mortem	59.00	1.88	61.69	2.49	ns
at sticking	38.48	3.59	42.61	3.99	ns
1 h post-mortem	28.42	2.15	36.08	3.81	0.1
T45 min SM (°C)	40.53	0.15	40.26	0.14	0.1
T45 min LD (°C)	40.54	0.12	40.08	0.23	0.1
pH45 min SM	6.27	0.13	6.26	0.06	ns
pH45 min LD	6.29	0.08	6.38	0.06	ns
Rigor mortis SM	7.2	1.2	6.4	0.5	ns
pH 22h	5.53	0.05	5.53	0.05	ns
T 22h (°C)	4.93	1.04	4.43	1.16	0.1
Fluid wt of filter paper, mg	43.1	8.3	42.7	8.7	ns
Drip loss in %	3.5	0.4	4.0	0.6	ns
Hunter L^*-value	54.30	0.99	55.24	0.58	ns
Hunter a^*-value	6.85	0.18	6.73	0.12	ns
Hunter b^*-value	12.92	0.26	13.37	0.21	ns

the Enriched housing pens had enough possibilities to explore in their home pen. Therefore, pigs from the Poor treatment may have been more motivated to leave their pen at loading. Results from a previous study (Geverink et al., subm.b) showed that pigs that were housed in poor conditions similar to those in the current study, were easier to load at slaughter if they regularly were given the opportunity to leave their pens, even when this was always followed by transport for 2 min in a transport box. Thus, it will take more time to load pigs from poor housing conditions if they have no previous experience with leaving their pen.

On the lorry, ambulation, time spent exploring the pen and time spent standing vs. sitting and lying did not differ between treatments, with the exception that Enriched pigs spent more time standing during the second half of transport. Salivary cortisol levels found in Poor pigs before and after transport were similar to levels found in previous studies in pigs kept in similar housing conditions (Geverink et al., subm.b). Surprisingly, cortisol levels in Enriched pigs were significantly higher both before and after transport. Higher baseline salivary cortisol levels in these pigs were found at 14 and 17 weeks of age, with the differences between Enriched and Poor pigs increasing with age (de Jong et al., 1998). De Jong et al. suggested that differences in HPA-axis activity or differences in the circadian rhythm of cortisol may have caused the differences in baseline cortisol. However, the cortisol levels they measured in Enriched pigs were on average 4 ng/ml, while levels measured here before transport were twice as high. The conditions on the morning of transport were different from those at other times, which may have increased the baseline cortisol levels. At 0600 the pens had been cleaned, but in contradiction to the normal procedure the enriched pens were not provided with fresh straw. Thus, on the moment of saliva sampling, the pigs had spent 3.5 h without straw. The lack of substrate otherwise available may have induced the large cortisol response observed. For example, it has been shown in other species that blood levels of corticosterone are increased in response to a low or zero reward for animals used to a large reward (Gray, 1987). Transport further increased salivary cortisol levels in Enriched pigs, showing that maximum stimulation of the adrenal cortex was not reached yet. The cortisol increase in Poor pigs was higher, suggesting that either transport induced a larger cortisol release in Poor pigs, or that maximum stimulation of the adrenal cortex in Enriched pigs was reached after transport. There is not much information available about the maximal salivary cortisol response of fattening pigs at slaughter weight to stress. Subsequent events of transport, driving and mixing resulted in salivary cortisol levels of ca. 32 nmol/l, which equals ca. 12 ng/ml, in more

extensively housed pigs (Geverink et al., 1998a). Exogenous ACTH stimulation in 100 kg gilts resulted in salivary cortisol levels of ca. 11 ng/ml in a study by Cook et al. 1996). However, comparing results from these studies may be confounding due to the different pig breeds and the different methods used to measure cortisol. Ruis (pers. comm.) reports that maximal stimulation of the adrenal cortex by an ACTH challenge resulted in maximum salivary cortisol levels of 10 à 15 ng/ml in 24 wk old pigs, using the same RIA as in the present study, but these animals were intensively housed.

In lairage, Poor pigs did not show as much exploratory behaviours as Enriched pigs. However, De Jong et al. (1998) showed that the Poor pigs showed the same amount of exploration as Enriched pigs during a social isolation test at 14 weeks of age and more exploration in a social confrontation test at 25 weeks of age. Others have found that pigs raised in barren environment show more exploration to a novel stimulus in their home pen (Stolba and Wood-Gush, 1980), or to novel stimulus in an open-door test (Pearce and Paterson, 1993). The different results found in this study could perhaps be attributed to the fact that preslaughter treatment, which involved loading, transport, and unloading in a new environment, is a stronger stressor then the tests in the other studies. For example, social isolation for one hour increased salivary cortisol in both Enriched and Poor pigs with 3 ng/ml, and heart rate returned to baseline immediately afterwards (De Jong et al., 1998). However, in this study loading and transport increased salivary cortisol levels with 6.68 ng/ml in Poor pigs and 3.63 ng/ml in Enriched pigs. In addition, transport is known to have a prolonged influence on heart rate and reduce exploratory behavior in lairage for pigs from intensive housing systems (Geverink et al., 1998b). Thus, besides a higher physiological response, preslaughter treatment may elicit a different behavioral response as well.

At sticking, plasma cortisol levels in Enriched pigs were also higher than levels in Poor pigs, though not significantly. Warris et al. (1983) found no differences in plasma cortisol at sticking either between pigs reared extensively under environmentally enriched conditions in an outside paddock or reared under intensive conditions in bare concrete pens. However, it should be noted that plasma cortisol concentrations represent the total, i.e. free and bound, hormone while salivary cortisol concentrations represent the 'free', i.e. the biologically active, hormone.

Glycogen content 1 h post-mortem showed a trend to be lower in pigs from Enriched pens, while levels did not differ at sticking or on the previous day. This suggests an enhanced post-mortem glycogen breakdown, but this did not result in differences in any of the meat quality variables measured. Several other studies showed

that free range pigs had similar meat quality and sensory characteristics compared to pigs raised intensively (Warriss et al., 1983; van der Wal et al., 1993). On the other hand, it has been observed that a lower incidence of DFD but a higher incidence of properties of PSE may occur in free-range pigs that have been exposed to preslaughter treatment that minimizes stress (Barton-Gade and Blaabjerg, 1989). The same trend was observed in pigs that were exposed to moderate exercise (Essén-Gustavsson et al., 1988; Enfält et al., 1993) or pigs that were regularly let out of their pen (Geverink et al., subm.b). It was suggested in these studies that free-range housing, moderate exercise and gentle preslaughter treatment may lead to increased glycogen levels before slaughter, and consequently to a reduced incidence of DFD, and maybe an enhanced post-mortem glycogen breakdown and increased incidence of PSE. However, in the present study the Enriched pigs did not differ from Poor pigs in glycogen content before slaughter.

Summarizing, pigs that were housed under moderately enriched housing conditions were more difficult to load than pigs from poor housing conditions, and this may be due to their previous experience with being outside their home pen. Enriched pigs displayed higher cortisol levels before and after transport, but Poor pigs showed a higher rise in cortisol. Because there were no differences in meat quality, and enriched conditions are known to have a beneficial influence on behaviour, enriched housing conditions should be preferred.

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Chapter 8

General discussion

Because most existing recommendations on the protection of animals during transport (EC Directive 1991; EC-working group 1992) and at the time of slaughter (EC Directive 1993) are not implemented in national laws yet, preslaughter treatment is not standardized and still likely to affect the welfare of pigs. Frequently used indicators of acute welfare problems are behavioural and physiological responses. Preslaughter treatment that evokes behavioural or physiological responses resulting in an increase in sympatho-adrenal or HPA-axis activity, increased exercise or elevated body temperature, is believed to lead to aberrant meat quality. In this thesis, we wanted to gain more insight in the relative contribution of common pre-slaughter treatment factors in evoking behavioural and physiological responses indicative of welfare problems. Therefore we subjected pigs to series of common preslaughter stressors, such as transport, driving, mixing, and slaughterhouse sounds. Furthermore, attention was paid to the effects of conditions during the fattening period on responses to preslaughter treatment, and to the consequences for meat quality.

Short-term transport

The common means of transporting pigs within Europe is the lorry. Within the Netherlands, the distance between farm and slaughterhouse is limited and transport duration will generally not exceed three hours. In the experiments described in this thesis, transport times varied between 25 and 45 minutes. All groups of pigs were transported unmixed, i.e. within their original group. Space allowance was approximately 0.6 m^2 per pig, which is higher than recommended space allowances (EC-working group, 1992). However, the latter densities do not result in optimal welfare since they are regarded as a compromise between animal welfare, meat quality and the economics of transport (Lambooij et al., 1995).

Pigs usually lie down after two to four hours of transport (Lambooij et al, 1995) and stand more on rough journeys compared to smooth journeys, which is suggested to alleviate the effects of travel sickness (Bradshaw et al., 1996a). In Chapter 7, we observed that most pigs stood during the 40 min transport. Our videotapes, which viewed the pigs from above, could not give certainty about the absence of travel sickness. Bradshaw et al. (1996c) travelled with pigs in the body of the lorry on a smooth journey and observed that half of the pigs began to exhibit behaviour associated with travel sickness after two hours which lasted for 2.5 h. Since our journey lasted only 40 minutes and can be categorised as a smooth journey, the chance for travel sickness to occur seems small.

The pigs spent on average 12% of their time exploring the holding pen on the lorry. This is considerably less than the time pigs spent after transport exploring a novel holding pen on the farm (on average 47%, Chapter 5) or in lairage (on average 40%, Chapters 6 and 7), indicating that exploratory behaviour is suppressed during transportation. However, it should be noted that the holding pen in the lorry was much smaller than those on the farm and in lairage, thus resulting in a higher density and offering less area to explore. Whether pigs originate from intensive or more extensive housing systems does not seem to influence their behaviour during transport (Chapter 7).

An increased heart rate during transport compared to previous resting levels has been described by various authors (Augustini and Fisher, 1982; Stephens and Rader, 1982; Villé et al., 1993). The experiment described in Chapter 5 shows that it is the actual transportation process itself, i.e. the motion of the vehicle, which is a major cause for an elevated heart rate. Pigs that were loaded on the lorry and transported for 25 minutes on a relative 'rough' journey (i.e. on minor roads) had a significantly higher heart rate than pigs that were loaded on the lorry which subsequently remained stationary for 25 minutes. In addition, during the post-transport period the transported pigs displayed less exploratory behaviors and spent more time lying, while their heart rate was lower than that of pigs from the stationary lorry. This shows that transportation exhausts the animals for a prolonged period.

Levels of salivary cortisol were significantly increased after transport (Chapter 3,5,6,7). The increase in all experiments was of the same magnitude, except for the smaller increase observed in pigs from enriched housing conditions in Chapter 7, for which the underlying cause remains to be elucidated. Several other authors have reported a stimulatory effect on cortisol release of short journeys (Becker et al., 1985; Nyberg et al, 1988; Zanella and Unshelm, 1994). In Chapter 5, it was shown that pigs that spent 25 minutes on a stationary lorry also showed elevated cortisol levels, but not as high as transported pigs. This indicates that the transportation process is responsible for a substantial part of the cortisol increase.

Pigs that were regularly let out of their pen and transported in a transport box during the fattening period did not differ in their cortisol response to transport from pigs that were regularly handled during the fattening period or pigs that did not receive either treatment (Chapter 6). However, the cortisol response to only the loading procedure may have been lower in the moved or handled pigs compared to control, with the subsequent stress of transport diminishing these differences.

It can be concluded on the basis of the behavioural, heart rate and cortisol data that the transportation process, i.e. the motion of the vehicle, has a negative impact on welfare. Thus, in practice measures should be taken to alleviate the stress associated with transport. Familiar recommendations are an optimal loading density and optimal ventilation (EC., 1992), and omission of mixing during transport (Bradshaw et al., 1996b). In addition, journeys should be as 'smooth' as possible, which implies careful driving on motorways. Vibration can also be reduced by a good suspension of the lorry. Research by Perremans et al. (1996) gives indications for maximum acceleration and frequency ranges of vibration in the vertical direction resulting in the least change in heart rate and stress hormones. A debatable method to reduce transport stress, which seems to be commonly applied in Belgium, is sedation with tranquillising drugs such as azaperone (Chapter 2). Azaperone exerts its effect by decreasing the release of catecholamines and exerting an α adrenergic blocking action (Gregory and Wilkins, 1986). Use of azaperone is not allowed in the Netherlands if pork is produced in accordance with the Integrated Quality Control (IKB) scheme (PVE, 1991). In other cases, the use is confined to Pietrain crosses (Hanssen, pers. comm.). The use of a magnesium compound included in the feed (Schumm, 1984) or electrolyte solutions containing potassium (Schaefer et al., 1997) have also been recommended to reduce transport stress. These compounds exchange for calcium ions in the muscle tissue and maintain the body's electrolytic system in a normal state. However, sedating the animals does not take away the cause of the problems. Clearly, the ideal situation would be if pigs did not have to be transported at all and could be slaughtered at the farm. Alternatively, it should be enforced by law that pigs should be transported to the most nearby slaughterhouse.

Moving and handling

Many fattening pigs have little experience of handling, especially during the final management stage before slaughter. Moving and handling pigs on the farm and in lairage during preslaughter treatment may therefore impose serious problems. Stockmen often use tools such as electric goads, boards and sticks to move pigs. Currently the use of electric goads is directed only for 'pigs which refuse to move, provided that shocks last no more than two seconds, are adequately spaced out and that the animals have room ahead of them to move' (EC, 1993). However, in practice all transport workers and slaughterhouse employees use electric goads to move pigs (Chapter 2), and are not likely to limit their use as decribed in the directive. Using

sticks to drive pigs should be discouraged as in Chapter 2 it was shown that sticks contribute considerably to skin damage in the hind region of the pig. Using boards is clearly the most welfare-friendly and easy method to move pigs because it minimizes bruises, and pigs that turn back are less likely to run past the handler with a board (Grandin, 1987).

The willingness to move can be increased by repeatedly letting pigs out of their pen during the fattening period, as was shown in Chapter 6. The treatment was applied during ten sessions, but the observed decrease in latency time to leave the pen during the course of the sessions suggests that two treatments may already lead to an effect. Pigs that were regularly handled during the fattening period were not easier to move than control pigs. This treatment was applied in order to reduce the fear of humans during handling. However, our Environment treatment may also have led to reduced fear. Hemsworth et al. (1996) showed that pigs associate a rewarding experience with the handler and this conditioning results in pigs being less fearful of the handler and other humans. In our Environment treatment, the opportunity to use a large area outside the holding pen can be viewed as a rewarding experience. This was initiated each time by a human opening the pen, and thus may have lead to reduced fear for humans. Eventually, the experience of having been outside the home pen seems to be the decisive factor in increasing the ease of moving. Furthermore, in Chapter 7 it was shown that pigs housed in intensive conditions were easier to move and load than pigs from more enriched conditions. Both groups of pigs had previous experience on three occasions with being let out of the pen for several tests. These events may have involved a positive component, i.e. the possibility to explore a new environment, for the pigs from intensive housing conditions, which showed less exploratory behaviours in their home pen than pigs from the enriched treatment. Thus, the most important aspect to increase the ease of moving of intensively housed pigs during preslaughter treatment is to accustom the animals to this procedure, and results from both Chapter 6 and 7 suggest that only a few sessions are needed to improve the willingness to move. It seems likely that the same effect can be accomplished in pigs from more enriched housing systems by using a positive treatment like the Environment treatment.

Individual differences between pigs within a group exist during handling, as some pigs are easier to move than others. However, these differences do not seem to be related to individual differences previously shown in the home pen (Chapter 3), and thus are not predictable from those.

Moving and handling pigs elevates both heart rate and cortisol levels. Heart rate during loading and unloading is higher than during transport, as was shown in Chapter 5, which is in agreement with findings by others (Augustini and Fisher, 1982). Climbing and descending the ramp of the lorry during loading will be a major cause for the observed increase in heart rate. Sympathetic activation during (un)loading can be reduced by using hydrolic lift pens (Augustini and Fisher, 1982), and during moving by careful handling (Troeger, 1989). In Chapter 5, it was shown that moving pigs out of their pen and loading them on a lorry which remained stationary resulted in elevated cortisol levels after 25 minutes. Bradshaw et al. (1996b) also found higher cortisol levels after 30 minutes for pigs loaded on a stationary lorry, but on both occasions part of the increase may be caused by the novel environment of the lorry. Unloading and moving pigs in the period after transport does not result in a further increase in cortisol levels compared to the already high levels measured immediately after transport (Chapter 3).

In conclusion, moving and handling will affect heart rate and cortisol levels particularly during (un)loading and thereby negatively affect welfare. The willingness to move can be significantly increased by regularly moving pigs during the fattening period. Consequently, pigs are less likely to be subject to rough handling and this may also reduce the physiological response to handling, thus having a beneficial effect on welfare.

Lairage

Pigs will usually be kept in a holding pen in lairage for a while to create a reservoir of animals, in order to maintain the constant speed of the slaughter line. A second function of lairage is to let the animals recover from transport. In Chapter 2 it was shown that in small slaughterhouses, the supply of pigs was sometimes insufficient for a number of hours, causing the number of pigs in lairage to be depleted. New pigs arriving at the slaughterhouse were then driven immediately to the stunning point without getting the opportunity to recover from transport. In the large slaughterhouses, pigs were kept usually for two hours in lairage. Whether pigs will actually get the opportunity in lairage to recover from transport and rest will depend mainly on whether they are mixed or not.

Mixing in lairage

The EC Directive on the protection of animals at the time of slaughter or killing (1993) states that 'animals which might injure each other on account of their origin must be kept and lairaged apart from each other'. However, it is still common practice to mix different rearing groups of a producer during transport and lairage, as was shown in Chapter 2. To avoid mixing of pigs, smaller holding pens would need to be created in the lorry and in lairage, and pigs would need to be kept within their group during the driving procedure. Apparently slaughterhouse directions feel that the positive effects of keeping animals within their own group do not outweigh the required efforts. However, results presented in this thesis show in agreement with other studies that mixing has a pronounced negative impact on welfare.

In the large groups of up to 90 animals fighting was shown to reach peak levels between 40-50 min and 60-70 minutes after introduction in the holding pen. Clearly, this does not stimulate resting behaviour. After 90 minutes in lairage, only one-third of the animals was observed to be lying down. When we simulated mixing in an experimental setting by mixing two groups of 6 or 7 pigs (Chapter 3), we observed that pigs mainly fought during the first half hour and rested during the second half hour. Also Moss (1978) found that the majority of aggressive encounters after mixing occur in the first 30 minutes when he mixed two lots of five pigs. A larger group size with relatively more unfamiliar pigs, which is common under commercial circumstances, may well prolong occurrence of fighting, which is detrimental to welfare.

The field study described in Chapter 2 showed large individual differences in involvement in agonistic encounters. In addition, in Chapter 3 it was observed that the frequency and duration of aggressive encounters during mixing were related to the level of aggressive behaviour sampled in the home pen, which suggests that certain individuals were predisposed to perform aggressive acts regardless of context. Furthermore, duration of fighting was related to the increase in cortisol.

In the survey it was shown that in Belgian slaughterhouses hardly any fights occurred because the animals were still drugged from treatment with azaperone prior to transport. Although a reduction in fights is an advantage, it should be stated that drugging the pigs does not take away the cause of the problems. Another solution that has been experimentally tested is the use of odour masking agents. Olfactory cues appear to play an important role in modulating aggression (McGlone, 1985). McGlone and Morrow (1988) were able to reduce aggression in pairs of prepuberal pigs by

spraying androstenone on the head and snout of pigs, while others found that the application of an odour masking agent during mixing had little effect (Friend et al., 1983; Lambooij, pers. comm.).

A period of rest in lairage is generally recommended to allow the pigs to recover from transport and the associated handling. It is believed that this will lead to the production of better meat (Tarrant, 1989) as well as being desirable from a welfare point of view (Warris et al., 1992). However, if animals are to be mixed, present results suggest that from the point of view of animal welfare, it may be better to slaughter the animals immediately upon arrival at the slaughterhouse.

Unmixed condition

If pigs are transported within their own group during transport and lairage, they do not fight (Bradshaw et al., 1996b; Chapter 7). A considerable percentage of time will be spend exploring the holding pen, in experimental circumstances (Chapter 5) as well as under commercial circumstances (Chapter 6 and 7). Spatial behaviour in the holding pen is influenced by exposing the animals to sound (Chapter 5). Pigs exposed to the sound of machines in lairage or white noise spent more time together with their group-mates in the same section of the holding pen than pigs that were not exposed to sound, with the pigs exposed to the sound of squealing pigs in lairage being intermediate. The sounds may expose a threat to the pigs to some extent although this is not reflected in elevated cortisol concentrations and heart rate. This suggests that staying close together with their conspecifics may help pigs to cope with sound stimuli. To test this hypothesis, the responses of individual pigs exposed to sound should be examined. In addition, further research could study whether pigs that are mixed in lairage and thus are not only surrounded by familiar conspecifics, have problems to cope with sound stimuli.

Consequences of pre-slaughter treatment for meat quality

Meat quality can be impaired by bruises and by the incidence of factors associated with PSE and DFD. Rough handling and fighting will cause bruises, thus reducing the carcass value (Tarrant, 1989). Using an electric goad to move pigs may increase blood spots in the meat (Calkins et al., 1980). In one of the Dutch slaughterhouses visited (Chapter 2), using sticks to move pigs contributed to skin damage in the hind region. Clearly, using a board to move pigs is the best method, to prevent bruises as well as from the welfare point of view.

Mixed pigs have more skin damage than unmixed pigs after lairage (Warriss and Brown, 1985; Karlsson and Lundström, 1992). In Chapter 3, it was shown that the increase in skin damage was positively correlated with duration of fighting. Under commercial circumstances, the longer animals get the opportunity to fight, the more skin damage they will have. This is supported by the results from the survey in Chapter 2, which showed that there was a significant association between lairage time and skin damage in the head and shoulder region. Mixing will also have an important influence on the incidence of PSE and DFD meat, because fighting will most likely lead to an increase in sympatho-adrenal and HPA-axis activity, increased exercise or elevated body temperature. Fighting due to mixing pigs in lairage has been shown to lead to both more PSE and DFD (Karlsson and Lundström, 1992). In the appendix of Chapter 3, it is shown that pigs that had higher cortisol levels after mixing and fought more during mixing, showed a higher pH 45 minutes post-mortem, which may be indicative of DFD.

When pigs are not mixed before slaughter, the reponses to transport and handling will become the most important variables with regard to meat quality. If transport and handling are done conform the recommendations (i.e. moving with boards, loading with hydrolic lift pens, 'smooth' journeys), it may be asked what other measures can be taken to avoid aberrant meat quality. One of the factors that may be of importance is the 'preparation' of the pigs for preslaughter treatment. In Chapter 6 and 7, an attempt was made to create conditions during the fattening period that resulted in the pigs being able to cope better with preslaughter treatment and thus leading to better meat quality. In Chapter 6, pigs were regularly given the opportunity to leave their pen and in addition were accustomed to transport in a transport box (Environment treatment). The Handling treatment involved the pigs being regularly handled within their home pen. The considerate treatment during handling and transport resulted in all treatment groups in meat with a relatively pale colour. Indeed, it has been suggested by other authors that pre-slaughter treatment that minimizes stress results in meat with properties of PSE (Barton-Gade and Blaabjerg, 1989). The underlying cause is thought to be a higher glycogen content in the muscles at the moment of slaughter (Nielsen, 1981; Monin and Sellier, 1985; Enfält et al., 1993). Thus, a considerate treatment on the day of slaughter may result in less DFD but more PSE (Tarrant, 1989).

Environment pigs showed paler meat with a lower waterholding capacity compared to control. The Environment treatment provided the pigs with moderate

exercise each time, and Environment pigs were faster to load on the lorry at the day of slaughter which was most likely associated with less physical activity. These factors may have contributed to higher glycogen levels pre-slaughter. When a considerate preslaughter treatment indeed leads to an increased chance for the development of PSE, it should be studied to what extent this can be counteracted by improvements in the method of carcass processing, e.g. chilling slows down the onset of rigor and also slows the rate of evaporative weight loss (Tarrant, 1989).

In Chapter 7, it was studied whether there were differences in muscle glycogen content between pigs that were raised in intensive versus more extensive housing systems, and subjected to the same considerate preslaughter treatment as in Chapter 6. To this extent, muscle biopsies were taken one day before slaughter, immediately after sticking and one hour post-mortem. Muscle biopsies were not taken during preslaughter treatment, because the study described in Chapter 4 showed that this procedure affects short-term behavioural and physiological variables. Results from the study showed that glycogen content 1 h post-mortem showed a trend to be lower in pigs from Enriched pens, but levels did not differ at sticking or on the previous day. The increase in space in the enriched home pen thus did not appear to lead to higher glycogen levels, as has been suggested by Barton-Gade and Blaabjerg (1989) for outdoor-pigs. There were no differences in any of the meat quality variables measured, which agrees with other studies (Warriss et al., 1983; van der Wal et al., 1993). This suggests that housing conditions during rearing and fattening are not an important factor with regard to meat quality.

Concluding remarks

The behavioural and physiological responses observed indicate that particularly transportation and mixing have a negative impact on welfare. Besides an optimal loading density and optimal ventilation during transport, journeys should be as 'smoothly' as possible, which implies careful driving on motorways. The ideal situation would be if pigs did not have to be transported at all and could be slaughtered at the farm. Alternatively, either pigs should be slaughtered immediately upon arrival at the slaughterhouse or legislation should prohibit to mix pigs during transport and lairage. Further research can be carried out to study the importance of social support from conspecifics during lairage to protect pigs from potentially adverse effects of exposure to lairage sounds.

Ideally, pigs should be raised and fattened in more extensive housing systems. In

addition, pigs should be regularly moved during the fattening period in order to increase the willingness to move during preslaughter treatment. Pigs that are easier to move are less likely to be subject to rough handling, which implies improved welfare, while the workload for stockmen is reduced. Future studies should estimate the minimum number of sessions needed to achieve this effect.

The factors mentioned above will improve welfare, but do not necessarily lead to improved meat quality. For example, meat from pigs that are easier to load due previous experience with moving show more properties of PSE, and considerate preslaughter treatment can also lead to impaired meat quality. The relationship between preslaughter handling, high energy reserves at slaughter and incidence of PSE merits further research. Opposite consequences for welfare and meat quality can lead to a dilemma while also economic and environmental considerations are inescapable aspects of issues concerning pork production. However, because an increasing group of consumers sets higher standards for animal welfare, the production of meat from pigs fattened and slaughtered under 'acceptable' conditions becomes of greater importance. When factors such as considerate preslaughter treatment leads to affected carcasses, it should be examined to what extent processing factors such as rapid chilling can reduce the expression of PSE.

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Summary

Chapter 1 is a general introduction to this thesis. Welfare of slaughter pigs may be impaired by preslaughter treatment. Frequently used welfare indicators are behavioural and physiological responses. The responses to preslaughter treatment may also have an effect on perimortem muscle metabolism and thereby on meat quality. Behavioural and physiological responses to acute stress could lead to properties of PSE (pale, soft, exudative) meat via an associated increase in muscle exercise, muscle temperature, or elevated hormone levels. Depletion of muscle glycogen by metabolic exhaustion will result in DFD (dark, firm, dry) meat.

The purpose of this thesis was to gain more insight in the relative contribution of common preslaughter treatment factors in evoking behavioural and physiological responses indicative of disturbed welfare. Furthermore, attention was paid to the effects of conditions during the fattening period on responses to preslaughter treatment, and to the consequences for meat quality.

Results from field observations on pigs in five Dutch and four Belgian slaughterhouses are described in **Chapter 2**. Brooms and/or electric goads were used to move pigs in all slaughterhouses and in one Dutch abattoir handling with sticks seemed to contribute considerably to skin damage. The level of agonistic behaviour was lowest during the first ten minutes in lairage. In the Dutch slaughterhouses, large individual differences in aggression between pigs were observed. Pigs in Belgian slaughterhouses were tranquillized prior to transport resulting in generally low levels of agonistic behaviour. In both the Dutch and the Belgian slaughterhouses, skin damage was higher in the front and in the middle region than in the hind region of the pig. For the Dutch slaughterhouses skin damage was associated with time kept in lairage and stocking density. It was suggested that to decrease aggression and skin damage and thus to increase welfare in the visited Dutch slaughterhouses, stocking density should be lower and pigs should be slaughtered as soon as possible after arrival.

The response of pigs to two simulated lairage events, driving and mixing, was investigated in an experiment described in **Chapter 3**. Five groups of six and five groups of seven 70-kg pigs were transported for 40 min on a lorry and subjected to one of the following treatments: two groups were driven down a passage; four groups were mixed for one hour (A and B together, C and D together); four groups received the driving treatment immediately followed by the mixing treatment ('combined treatment'). Initial transportation led to increased concentrations of cortisol. Behaviour during driving was

not correlated with concentrations of cortisol after driving and cortisol did not increase relative to post-transport levels. Frequency and duration of fighting during mixing were positively correlated with aggression in the home pen and increase in concentrations of cortisol during mixing. One hour after the start of mixing, concentrations of cortisol had decreased relative to post-transport levels. After the combined treatment, all correlations described for the mixing treatment were absent and concentrations of cortisol increased relative to post-transport levels. Skin damage was highest after the mixing treatment. The responses observed indicate that the combination of driving and mixing, which is very common in lairage, leads to a greater response than in the case of each individual treatment. In the Appendix of Chapter 3, it is described that pigs that had high cortisol concentrations after mixing and fought more during mixing, had a higher pH 45 min post-mortem, which is indicative of DFD.

Chapter 4 describes behavioural and physiological responses of slaughter pigs to the shot biopsy, a method commonly used to study muscle tissue processes and predict meat quality. From 10 23-wk old gilts, one sample from the longissimus muscle was obtained using a cannula connected to a captive bolt. Ten other gilts were used as a control and received a sham shot. One week later, a second shot biopsy was taken from the experimental group. Behavioural and salivary cortisol responses to both biopsies were the same. All pigs flinched in response to the biopsies. Salivary cortisol was increased after 15 min. In both tests heart rate increased in response to the presence of the technician. In response to the first biopsy heart rate increased but not in response to the second biopsy. The experimental pigs showed a decrease in initiating contact with the technician in the second test. It is concluded that shot biopsy had a significant acute effect on behavior and physiology. Therefore, the usefulness of the technique in studies on the relation between pre-slaughter stress and meat quality is limited.

In **Chapter 5**, the behavioural and physiological responses of pigs to transport and subsequent exposure to slaughterhouse sounds were examined. Fourty-one groups of four slaughter pigs were separately loaded onto a lorry and transported for 25 min. Another 43 groups were loaded onto the lorry which then remained stationary for 25 min. Following unloading pigs were moved to a novel holding pen. Either one of the following sounds was played at 85 dB(A) for 10 min: Squeals of pigs in front of the restrainer, Machines in lairage, White noise, or Control (no sound). Heart rate was higher on the lorry for transported groups. In contrast, heart rate was lower during unloading, the

sound exposure period and the post-sound period for transported groups compared to stationary groups. Salivary cortisol concentrations were higher after transport than after the stationary period and remained higher for transported pigs after the sound exposure period. Transported pigs spent less time exploring the race and were less active than pigs from the stationary lorry. Pigs exposed to the Machines and White noise treatment spent more time close to their group-mates compared to Control pigs, with pigs subjected to the Pig sound being intermediate. Heart rate and cortisol levels did not differ significantly between sound treatments. It is tentatively suggested that social support from conspecifics may protect pigs from potentially adverse effects of exposure to lairage sounds.

Chapter 6 studies the effects of regular moving and handling during the fattening period on behavioural and physiological responses of pigs during preslaughter treatment, and consequences for meat quality. From the age of 10 wk onwards, 144 pigs were housed in groups of four and subjected 10 times to one of the following treatments. The Environment treatment allowed pigs to move freely outside their home pen and in addition accustomed the pigs to transport in a box. In the Handling treatment, pigs were handled by an experimenter in the home pen. A Control treatment was also included whereby the pigs were subjected to no treatment. During preslaughter treatment, the stockmen needed less time to move Environment pigs out of their pen and into the transport box. There were no differences between treatments in salivary cortisol concentrations before or after transport. Environment and Handling pigs had paler meat than Control pigs. Glycogen content at 1 h post-mortem and waterholding capacity were lower in Environment pigs compared to Control, but this did not lead to a higher incidence of PSE-meat. It is concluded that those pigs that had experience with leaving their home pen were much easier to handle at loading. Pigs that are easier to move are less likely to be subject to rough handling, which implies improved welfare, while the workload for stockmen is reduced. Differences in meat quality due to treatment were slight.

In **Chapter 7**, the effects of moderately enriched housing conditions on behavioural and cortisol responses during preslaughter treatment are described, as well as the consequences for meat quality. A total of 48 slaughter pigs were either raised in intensive housing conditions ("Poor" treatment: standard farrowing crates followed by standard rearing and fattening pens) or in more extensive conditions ("Enriched" treatment: larger farrowing pens followed by larger rearing and fattening pens, all provided with straw).

During preslaughter treatment, stockmen needed significantly less time to load pigs from Poor housing conditions. Enriched pigs showed higher salivary cortisol levels before transport, but the cortisol increase in response to transport was higher in Poor pigs. Enriched pigs showed more exploratory behaviours in lairage. Glycogen content 1 h post-mortem showed a trend to be lower in pigs from Enriched pens, while levels did not differ at sticking or on the previous day. There were no differences in any of the meat quality variables measured.

It is discussed in **Chapter 8** that the behavioural and physiological responses observed indicate that particularly transportation and mixing have a negative impact on welfare. Journeys should be as 'smoothly' as possible, which implies careful driving on motorways. Either pigs should be slaughtered immediately upon arrival at the slaughterhouse or legislation should prohibit to mix pigs during transport and lairage. Further research can be carried out to study the importance of social support from conspecifics during lairage to protect pigs from potentially adverse effects of exposure to lairage sounds.

The willingness to move during preslaughter treatment can be significantly increased by regularly moving pigs during the fattening period. Future studies should estimate the minimum number of sessions needed to achieve this effect.

The relationship between preslaughter handling, high energy reserves at slaughter and incidence of PSE merits further research. When considerate preslaughter treatment leads to affected carcasses, it should be examined to what extent processing factors such as rapid chilling can reduce the expression of PSE.

Samenvatting

De voorwaarden waaraan de behandeling van slachtvarkens tijdens het transport naar, en tijdens het verblijf op het slachthuis moeten voldoen zijn vastgelegd in verscheidene Europese richtlijnen. Deze richtlijnen zijn echter nog niet geïmplementeerd in de wetgeving. Het gevolg hiervan is dat de behandeling van slachtvarkens nog niet gestandaardiseerd is, waardoor het risico bestaat dat het welzijn van de dieren geschaad wordt.

Om een indruk te krijgen van de welzijnsproblemen bij varkens als gevolg van de behandeling op de slachtdag, zijn met name gedragsresponsen en stressfysiologische responsen bruikbare indicatoren. Een dier waarvan het welzijn verstoord is, zal minder normaal gedrag, zoals exploratie, vertonen. Een angstig dier zal niet willen lopen, of proberen te ontsnappen. Gedragingen die indicatief zijn voor pijn en ziekte zijn bijvoorbeeld braken, trillen en moeilijk lopen. Belangrijke indicatoren tijdens transport zijn rustgedrag en positieverandering. Wanneer groepen varkens afkomstig uit verschillende hokken tijdens transport en op het slachthuis samen gehuisvest worden (het zogenaamde 'mengen'), gaan de dieren meestal vechten om een nieuwe rangorde vast te stellen. In zo'n situatie kan bijvoorbeeld naar de frequentie en duur van agressief gedrag gekeken worden. De belangrijkste fysiologische indicaties van stress zijn een veranderde activiteit van het sympatho-adrenerge-systeem, te meten aan bijvoorbeeld een veranderde hartslag, en een veranderde activiteit van de hypothalamus-hypofyse-bijnieras, te meten aan bijvoorbeeld het niveau van het bijnierschorshormoon cortisol in het bloed of in het speeksel.

De hierboven beschreven gedrags- en fysiologische responsen kunnen leiden tot een verandering in het spiermetabolisme rondom het tijdstip van slachten. Dit kan uiteindelijk resulteren in een slechte vleeskwaliteit. De bekendste afwijkingen in vleeskwaliteit zijn het zogenaamde PSE-vlees (pale, soft, exudative oftewel bleek, slap, nat vlees) en het DFD-vlees (dark, firm, dry oftewel donker, hard, droog vlees). DFD-vlees ontstaat wanneer een varken gedurende langere periode aan stress is blootgesteld waardoor de energievoorraad in de spieren, het glycogeen, opraakt. Stress vlak voor de slacht kan via een grotere fysieke en fysiologische activiteit leiden tot een verhoogde post-mortem afbraak van glycogeen terwijl de spieren nog warm zijn, wat uiteindelijk kan resulteren in PSE vlees.

Het doel van het onderzoek dat beschreven staat in dit proefschrift, is om meer inzicht te krijgen in de relatieve bijdrage van specifieke behandelingsfactoren (transport, opdrijven, mengen, en blootstelling aan slachthuisgeluiden) op de gedrags- en fysiologische responsen van varkens. Verder wordt aandacht besteed aan de invloed

van huisvestings- en behandelingsfactoren op de boerderij op deze responsen, en aan consequenties voor de vleeskwaliteit.

In hoofdstuk 2 worden de resultaten van een studie naar de dagelijkse praktijk in vijf Nederlandse en vier Belgische slachthuizen beschreven. In alle slachthuizen gebruikten de werknemers bezems en/of prikkelaars om varkens op te drijven. In een van de Nederlandse slachthuizen gebruikte het personeel elastische stokken om de varkens op te drijven, waardoor de dieren veel huidbeschadigingen opliepen. In alle slachthuizen was het gebruikelijk om varkens in de wachtruimte te mengen in groepen van ca. 30 tot 90 dieren. Dit leidde tot een toenemend aantal gevechten tussen varkens met een piek in agressie na een tijdsduur van ca. 1 uur. Binnen een groep waren grote individuele verschillen in agressie te zien; een paar dieren waren de grootste vechters terwijl ca. 40 procent van de dieren helemaal niet bij gevechten betrokken was. In de Belgische slachthuizen vertoonden de varkens nauwelijks agressief gedrag, omdat ze voorafgaand aan het transport met een tranquilizer ingespoten waren. In zowel de Nederlandse als de Belgische slachthuizen waren er meer huidbeschadigingen op de kop en de romp van het karkas dan op de ham te zien. In de Nederlandse slachthuizen hing de mate van huidbeschadiging af van de tijdsduur die het dier in de wachtruimte had doorgebracht, en van het aantal dieren per vierkante meter in de wachtruimte.

Om gevechten tussen varkens te vermijden en zo de mate van huidbeschadigingen terug te dringen, zou de dichtheid in de wachtruimte dus zo laag mogelijk moeten zijn. Verder zouden de varkens niet gemengd moeten worden in de wachtruimte, óf ze zouden zo snel mogelijk na aankomst op het slachthuis geslacht moeten worden.

Hoofdstuk 3 behandelt de reacties van varkens op blootstelling aan twee gesimuleerde slachthuisaspecten, het opdrijven en het mengen. Groepjes van 20 weken-oude varkens werden eerst 40 min getransporteerd en vervolgens onderworpen aan een van de volgende behandelingen: 1) opdrijven over een circuit over een afstand van 148 m, 2) 1 uur mengen met een andere groep, en 3) een combinatie van beide behandelingen. Het transport resulteerde in verhoogde cortisolconcentraties in het speeksel. Het opdrijven verhoogde deze cortisolconcentraties niet verder. De volgorde binnen de groep tijdens het opdrijven correleerde niet met de cortisolconcentraties na afloop van het drijfproces. Nadat de varkens een uur gemengd waren, namen de cortisolconcentraties af ten opzichte van de concentraties na transport, maar waren

nog wel hoger dan de concentraties voor transport. De duur van de gevechten tijdens het mengen was positief gecorreleerd met 1) de agressie die de varkens vertoonden in hun eigen groep op de leeftijd van 17 weken 2) de verhoging van de cortisolconcentraties tijdens het mengen, en 3) de opgelopen huid-beschadigingen tijdens het mengen. Bij de gecombineerde behandeling waren deze correlaties echter niet significant. De gecombineerde behandeling leidde wel tot verhoogde cortisolconcentraties ten opzichte van de concentraties na transport. De combinatie van opdrijven en mengen, die in de praktijk bijna altijd voorkomt, veroorzaakte dus een hogere cortisolrespons dan de individuele behandelingen.

In de appendix bij hoofstuk 3 wordt vermeld dat de varkens die meer vochten tijdens het mengen en hogere cortisolniveaus na het mengen hadden, 45 min post-mortem een hogere pH hadden in de rugspier, wat indicatief kan zijn voor het ontstaan van DFD-vlees.

Hoofdstuk 4 beschrijft de gedrags- en fysiologische responsen van slachtvarkens op het nemen van een spierbiopt. Spierbiopten van slachtvarkens worden vaak gebruikt om de groei van het spierweefsel te bestuderen en ook om de vleeskwaliteit te voorspellen. Het gebruik van spierbiopten zou nuttig kunnen zijn in het onderzoek naar de relatie tussen stress en vleeskwaliteit, maar dan moet het nemen van een spierbiopt zélf geen invloed hebben op de gedrags- en fysiologische indicatoren die in het stressonderzoek gebruikt worden. Om dit uit te zoeken nam een biotechnicus bij 23-weken oude varkens een spierbiopt van ca. 60 mg uit de rugspier. Hiervoor gebruikte hij een biopsie-apparaat, bestaande uit een schietmasker met daarop een holle naald. Een week later werd een tweede biopt bij dezelfde dieren genomen. De varkens vertoonden vergelijkbare veranderingen in gedrag en in cortisolniveaus als reactie op beide biopten. Alle dieren vertoonden een reflex (ze maakten een klein sprongetje van de biotechnicus weg). De cortisolconcentraties in het speeksel waren 15 min na de bioptname verhoogd. De hartslag van de dieren ging in de beide testen omhoog vanaf het moment dat de biotechnicus in het hok stapte en de voorbereidingen voor het biopt ging nemen. Na de eerste bioptname ging de hartslag nog verder omhoog, maar in de tweede test bleef een verdere verhoging achterwege. In de tweede test zochten de varkens minder contact met de biotechnicus terwijl deze de voorbereidingen op het biopt nam, wat erop wijst dat ze het eerste biopt waarschijnlijk als onprettig ervoeren.

De bioptname had dus een effect op gedrag en fysiologie. Daarom is de

bruikbaarheid van deze techniek in studies naar de relatie tussen stress en vleeskwaliteit beperkt.

In hoofdstuk 5 worden de gedrags- en fysiologische responsen van varkens op transport en daaropvolgende blootstelling aan slachthuisgeluiden onderzocht. Groepen van vier varkens werden op een transportwagen geladen. Vervolgens werden ze ófwel 25 min getransporteerd, ófwel 25 min op de stilstaande wagen gehouden ('stationaire' behandeling). Op deze manier kon het effect van het transport zelf, dus het rijden van de wagen, gescheiden worden van het effect van het opladen en van het verblijf in de onbekende omgeving van de wagen. Nadat een groep varkens weer was uitgeladen, werden de dieren naar een voor hun onbekende stal gebracht. Hier werden ze blootgesteld aan één van de volgende geluidsopnames die op 85 dB(A) werden afgespeeld: 1) gegil van varkens in het slachthuis, 2) machines in het slachthuis, 3) witte ruis (geluid waarbij alle frequenties hetzelfde geluidsdrukniveau hebben), of 4) controle (geen geluid).

De varkens die getransporteerd werden vertoonden een hogere hartslag tijdens het verblijf op de wagen dan de varkens in de stilstaande wagen. Tijdens het uitladen en de geluidsperiode was de hartslag van de getransporteerde varkens echter lager dan de hartslag van de stationaire varkens. De getransporteerde varkens vertoonden na afloop hogere speekselcortisolconcentraties dan de stationaire varkens. De getransporteerde varkens waren minder actief tijdens de geluidsperiode en vertoonden minder exploratief gedrag. Deze resultaten geven aan dat het verblijf op de rijdende wagen een langdurig effect had op gedrag en fysiologie, en de dieren uitputte.

Tussen de vier geluidsbehandelingen waren geen verschillen waar te nemen in hartslag of cortisolconcentraties. Varkens die blootgesteld werden aan het geluid van de machines of aan de witte ruis bleven echter dichter bij elkaar dan de controlevarkens. De varkens die blootgesteld werden aan het geluid van de gillende varkens bleven ook wel dichter bij elkaar dan de controlevarkens, maar dit was geen significant verschil. De resultaten suggereren dat de varkens die blootgesteld werden aan geluid sociale steun van soortgenoten opzochten.

Hoofdstuk 6 behandelt de invloed van de omstandigheden op de boerderij op gedragsmatige en fysiologische veranderingen bij varkens op de slachtdag en de consequenties voor de vleeskwaliteit. Vanaf een leeftijd van 10 weken ondergingen varkens in totaal 10 keer één van de volgende behandelingen. In de 'Environment'-

behandeling werd de deur van het hok opengezet zodat de dieren in een gedeelte van de gang voor het hok konden komen. Vervolgens werden ze in een transportbox gezet en binnen de stal rondgereden. In de 'Handling'-behandeling verbleef een onderzoeker gedurende een korte periode in het hok, aaide de dieren, liep rond in het hok en pakte alle dieren ook even stevig bij de nek beet. Varkens in de controlebehandeling ondergingen geen van beide behandelingen.

Op de slachtdag had het boerderijpersoneel minder tijd nodig om de Environment-varkens uit hun hok te halen en op te laden. Er waren geen verschillen tussen behandelingen in cortisolconcentraties in het speeksel voor of na transport. Na slacht was het vlees van de Environment- en Handling-varkens bleker dan dat van de controlevarkens. Vergeleken met de gangbare vleeskleur was echter ook het controlevlees bleek. Rugspierbiopten van Environment-varkens, die 1 uur post-mortem genomen waren, bevatten minder glycogeen dan biopten van controlevarkens. Ook was het waterbindend vermogen van hun vlees slechter dan van de controlevarkens, maar al deze factoren resulteerden niet in een hogere incidentie van PSE-vlees bij de Environment-behandeling.

De varkens die op de boerderij regelmatig buiten hun hok waren geweest, waren dus veel makkelijker te hanteren tijdens het opladen op de slachtdag. Varkens die makkelijker te laden zijn, zullen minder snel ruw opgedreven worden door het personeel. Dit komt het welzijn van deze varkens ten goede, terwijl het personeel minder zwaar werk hoeft te doen.

In hoofdstuk 7 worden de effecten van huisvesting op de boerderij op de respons op behandeling voor de slacht bestudeerd. Uit ander onderzoek blijkt dat varkens die in extensieve systemen gehuisvest worden, minder agressief gedrag en abnormaal gedrag zoals staartbijten vertonen dan varkens in intensieve huisvestingssystemen. In dit experiment werden varkens gehuisvest in intensieve systemen (0.84 m^2 per dier, geen stro) of in meer extensieve systemen (1.16 m^2 per dier, met stro). Tijdens deze periode werden de varkens drie keer uit hun hok gehaald om getest te worden binnen een ander onderzoeksproject.

Op de slachtdag had het personeel minder tijd nodig om de intensief gehuisveste varkens op te laden. Waarschijnlijk omdat de intensieve varkens in hun hok minder mogelijkheden tot exploratie hadden dan extensieve varkens, en doordat de drie keer dat ze uit hun hok gehaald werden een verrijking voor de intensieve varkens betekenden, waren ze meer gemotiveerd om hun hok te verlaten dan extensieve

varkens. Extensieve varkens hadden hogere speekselcortisolniveaus voor en na transport, maar intensieve varkens vertoonden een hogere relatieve stijging in cortisolniveau's. De glycogeenconcentraties in spierbiopten, die één dag voor slacht en direct na het slachten uit de rugspier waren genomen, verschilden niet tussen de twee behandelingen. Een uur post-mortem was de glycogeenconcentratie echter lager in biopten van extensieve varkens, wat wijst op een verhoogde glycogeenafbraak. Er was echter geen aantoonbaar verschil in vleeskwaliteit tussen de twee types varkens.

Uit de discussie in hoofdstuk 8 blijkt dat uit de gedrags- en fysiologische metingen kan worden afgeleid dat met name transport en mengen een negatief effect hebben op het welzijn van varkens. Transport zou daarom zo rustig mogelijk moeten verlopen. De wetgeving zou het mengen van varkens tijdens transport en op het slachthuis moeten verbieden, óf varkens zouden meteen na aankomst op het slachthuis geslacht moeten worden.

Er zou meer onderzoek moeten worden uitgevoerd om het belang van sociaal contact met soortgenoten op het slachthuis te bestuderen.

Door varkens regelmatig uit hun mesthok te laten, zijn ze op de slachtdag makkelijker op te drijven. Er zou nader onderzoek moeten worden gedaan om vast te stellen hoeveel sessies tenminste nodig zijn om het gewenste effect te bereiken.

Het verband tussen de behandeling voor de slacht, hoge glycogeen-concentraties op het moment van slacht en de incidentie van PSE vereist meer onderzoek. Een rustige behandeling voor de slacht kan een positief effect hebben op het welzijn van varkens maar een negatief effect op de vleeskwaliteit. Aangezien een steeds grotere groep consumenten veel waarde hecht aan diervriendelijke vleesproductie, is het van belang om 'varkens af te mesten en te slachten onder welzijnsvriendelijke condities. Wanneer een rustige behandeling voor de slacht toch leidt tot een slechtere vleeskwaliteit, moet worden uitgezocht in hoeverre in de vleeslijn factoren zoals het koelen van karkassen het voorkomen van PSE kunnen reduceren.

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