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Pre-slaughter stress and pork quality

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Abstract. Stress is an inevitable consequence of handling of animals for slaughter. Stress conditions during transport, lairage and at slaughter induce undesirable effects on the end quality of meat such as pale, soft, exudative meat and dark firm dry meat. Hence, it is very important to define appropriate parameters for objective assessment of level of stress. Attempts to define measures of stress have been difficult and no physiological parameter has been successfully used to evaluate stress situations. One physiological change in swine associated with animal handling stress and with pork quality is an increase in blood lactate concentration. Plasma cortisol was thought to be an appropriate indicator of stress, but the concentration was not consistently changed by different stressors. Therefore, finding alternative parameters reacting to stressors, such as acute phase proteins, would be of great value for the objective evaluation of level of stress and meat quality. As the stress during pre-slaughter handling is unavoidable, the final goal is to improve transport and slaughter conditions for the animal and, as a consequence, meat quality and animal welfare.

1. Introduction

The handling of animals for slaughter consists of a series of procedures that are unusual for them and, therefore, stressful. Stress conditions during transport, lairage and at slaughter negatively influence meat quality. Bleeding interrupts blood circulation and oxygen supply to the muscle. Under these anaerobic conditions, the breakdown of glycogen/glucose results in an accumulation of lactic acid and induces the progressive acidification of the muscle, denaturation of muscle protein and the conversion of the muscle into meat [1]. If stressful conditions occur immediately prior to slaughter, the presence of high lactic acid concentration reduces muscle pH within the first hour after slaughter, while carcass temperature is still high. The combination of low pH and high temperature in the meat causes the denaturation of some muscle proteins leading to reduction in their water holding capacity and to changes of the colour [2]. Meat becomes pale, soft, and exudative (PSE) [3]. On the other hand, when animals are exposed to chronic or long term stress before slaughter, glycogen is depleted and less lactic acid will be formed post-mortem. At high pH value, relatively few proteins are denatured, so the water is firmly bound, and little or no exudates are formed [1] leading to the occurrence of dry, firm and dark meat (DFD).

The objective of this study was to evaluate the effect of stress induced with pre-slaughter handling on pork quality as well as to evaluate parameters for assessing stress level.

2. Assessment of meat quality defects

To identify meat quality defects, different parameters are used: pH, temperature, drip loss, colour and electrical conductivity. There are no unique standards for assessment of PSE or DFD meat. PSE meat is commonly defined as having a pH at 45 min after slaughter (pH45) lower than 6 [4]. If the drip loss is greater than 5%, that meat can be classified as PSE [5,6,7]. The pH at 24 h (pH24) alone can be

used to assess DFD meat. A pH24 greater than 6.0 is related to DFD meat [1]. pH24 greater than 6.2 is related to a serious DFD problem [8]. One of the most important components in physical appearance is colour, which the consumer uses as an indicator for the quality and freshness of the meat [9]. An optimal range of visual colour, measured subjectively according to a reference colour scale, for meat would be around 3-4, but values lower than 3 or higher than 4 would be considered PSE and DFD meat, respectively [4]. For assessing PSE, some authors have proposed a combination of parameters [5,7].

3. Pre-slaughter handling

Meat quality is influenced by multiple interacting factors which include breed, genotype, feeding, pre-slaughter handling, stunning, and slaughter method, chilling and storage conditions. Pre-slaughter handling consists of handling the animals both on the farm and during the transport, at lairage, and finally on their way to be stunned and slaughtered. These handling practices can all induce stress either psychologically or physically and are known to be responsible for the development of aberrant pork quality.

3.1. Transport

The impact of transport on animal welfare must be seen as a multiple challenge, for which a combination of stress factors is responsible for the welfare of animals. Stress caused by transport can result in pig fatigue, injury, poor meat quality and ultimately death [10]. The factors during transport that may compromise pig welfare are loading and unloading, journey duration and ambient temperature, placement on the transporter, stocking density, vibrations, floor type and bedding, mixing animals from different groups and food and water deprivation [11]. The interaction of these factors, plus the time spent in lairage and handling of pigs, makes it difficult to assess the impact of transport on pork quality. The relationship between journey length and transport stress does not appear to be linear [10,12]. However, short transport (<2 h) can cause acute stress when the level of glycogen is still high, and therefore the occurrence of PSE meat, while longer transport (>2 h) can exhaust glycogen depots in the muscles, causing the occurrence of DFD meat [10].

3.2. Lairage

Besides creating a reservoir of animals aimed at maintaining the constant speed of the slaughter line, the function of lairage is to allow the animals to recover from the stress of transport and unloading [13]. When pigs are subjected to highly stressful lairage conditions, lairage can have an additive effect to transport stress, and pigs can still be stressed at slaughter and produce poor pork quality [14]. Inadequate treatment of slaughter pigs in this stage, mixing unfamiliar pigs, pen size, stocking density and floor type and lairage temperatures and humidities can result in additional stress leading to skin damage and poor meat quality. Therefore, proper resting time is very important to relieve stress and improve meat quality. The effects of different lairage times on both animal welfare and meat quality are not well defined. Shorter lairage is associated with more PSE meat, because of insufficient time to relieve stress. Longer lairage can increase the amount of DFD meat and reduce carcass yield [15].

3.3. Race to the stunning chamber and stunning methods

Pre-stunning handling facilities are of primary importance, given the need to handle pigs faster, so as to follow the speed of the slaughter-line. The combination of higher speeds of slaughter lines, poorly designed animal handling systems and the size of the group in the depot affect the welfare of animals and the pork quality, as it increases the use of electric prods, which does not always lead to speeding up pigs coming to the stunner. Indeed, the use of electric prods increases mounting behaviour between pigs in the group, resulting in more fatigued pigs and a higher proportion of bruised carcasses and PSE meat [13].

In order to reduce pain and promote the welfare of animals during slaughter, a series of stunning methods have been designed and described, including electric stunning and stunning with carbon

dioxide (CO_2). Electric stunning requires the animal to be restrained, which is a potential stressor. Additionally, increased physical stress just prior to electric stunning and tonic and clonic convulsions accelerate post-mortem glycolysis, leading to the occurrence of PSE meat. During CO_2 stunning, the pigs are moved into the stunning chamber in groups using their natural group behaviour [11]. The development of these systems reduced the stress before slaughter and, consequently, the appearance of PSE and DFD meat.

4. Assessment pre-slaughter handling

During pre-slaughter handling of pigs, they react to different stressors which can be classified as physical or psychological [16]. The psychological state of animals can only be indirectly assessed, by monitoring behaviour and by measuring physiological parameters, such as level of cortisol and catecholamines from plasma (adrenalin and noradrenalin), given that stressful situations increase the concentration of these hormones [17]. Behavioural measurements usually represent adaptive responses to the environment (exploration, flight, immobilization, aggression, etc.) [18]. Behavioural, physiological and metabolic responses to aversive situations, besides the type, duration and intensity of the individual pre-slaughter stressor, depend on genetic background and prior experience of the animals [18]. Therefore, the assessment of the situation and the resulting stress is subjective, that is, dependent on the individual [18]. Differences in the sensory quality of pork can be, at least partly, explained by differences in an animal's reaction to stress as well as in the effect of these reactions on muscle glycolysis [19,20].

4.1. Physiological parameters of stress

4.1.1. Cortisol. Numerous experimental results indicate an increased level of cortisol in pigs caused by stress on day of slaughter, stress just before slaughter and physical activity [20,21,22]. Secretion of cortisol is highly variable and different factors must be taken into account, such as the time elapsed from stress to sampling, variation of concentration due to diurnal secretion, genetics and effects of chronic stress [23]. In addition, the concentrations of cortisol do not correspond to the stress intensity, so only the exposure of the pigs to the new environment is sufficient to increase its concentration to the maximum level [20]. Although several studies have investigated the association between cortisol concentration and meat quality, this is still a topic of debate. Some studies indicate that concentration of cortisol had no effect on the pork quality [22], while others suggest that increased concentrations of cortisol lead to the decrease in pork quality [20]. Moreover, measurement of cortisol levels is not very informative for the detection of chronic stress situations, and in this regard, may not be a good indicator of meat quality and sensory quality in pigs [20].

4.1.2. Lactate. Level of lactate can be a good indicator of physical and psychological stress in pigs. Blood lactate concentration was used for assessing pre-slaughter handling [21], stunning pigs with different concentrations of CO_2 [24], transport [25] and time spent in lairage and different plants [26]. Exsanguination blood lactate changes with physical activity, frequency of the use of electric prods and vocalization [27]. Increase in blood lactate concentration, which is associated with pre-slaughter stress, has been shown to have a negative effect on pork quality. Stress was associated with high concentrations of exsanguination blood lactate and lower meat quality such as decreased water holding capacity, lighter colour [14] and lower pH45 value [28].

4.1.3. Acute phase proteins. Finding alternative stress biomarkers is of great importance for the objective assessment animal welfare and optimization of production systems. Acute phase proteins (APP) are plasma proteins considered to be markers of inflammation, primarily synthesized as part of the acute phase response (APR) [29]. They also have been proposed as indicators for farm animal stress monitoring [30,31]. Stress caused to the animals during transport, the new accommodation and the pre-slaughter handling affects the change in concentration of PAF [32]. Some APPs react to a

lesser extent than others to the same stimulus and can also react differently to different types of stimuli [33]. Therefore, the use of more than one APP is proposed in assessment of stress levels. Given that moderate PAF increases its concentration only two to three times during the response, the major PAFs, which increase their concentration ten to one hundred times, are more interesting for assessing the health and welfare of pigs. In swine, among others, major APP are pig-MAP (Major Acute-phase Protein), C-reactive protein (CRP), haptoglobin (Hp) and serum amyloid A (SAA) [29,34]. Hp is the most widely studied PAF in pigs, mainly due to the availability of methods for determining its concentration. Increased levels of pig-MAP and Hp are linked with stress situations such as transport [35], crowding, mixing unfamiliar pigs [36, 37], or an inadequate handling of feed [34]. Pig-MAP was the most sensitive protein in the detection of the stress caused by changes in the feeding pattern [34], in distinguishing healthy from diseased states [33] and was the only APP which showed concentrations changed in pigs housed at different stocking densities [38]. Pig-MAP has advantages over other PAFs, such as Hp, due to a lower degree of variation its basal concentration, which facilitates the establishment of limits for distinguishing normal from pathological states and stress situations [32]. The concentration of SAA increases within four hours and the maximum is reached within 24 to 48 hours after a triggering event [33]. Therefore, this PAF can be used to evaluate novel situations or to evaluate pigs' reaction to environmental change, if the appropriate period between the stressful situation and the sampling is adopted [39]. Elevated levels of saliva SAA are a good marker of short transport stress (physiological-psychological stress) and social isolation (psychological stress) in pigs [39]. SAA is more susceptible to acute rather than chronic inflammation [33], which raises issues related to the sensitivity of this APP to high stress situations involving the use of electric prods and consequently, the appearance of skin damage.

5. Conclusions

Pre-slaughter stress has negative effects on pork quality. Minimizing this stress is important, not only for animal welfare, but also for improving pork quality. Stress reactivity is an individual characteristic and each pig is characterized by an individual specific range of values, within which the stress parameters can vary. There is no consistent association between the stress parameters and meat quality measurements. The relationship between cortisol levels and stress, as well as between cortisol levels and meat quality parameters is not linear. Therefore, it is important to determine the appropriate parameters for assessing the level of stress. Lactate concentrations showed good correlation both with pre-slaughter stress and meat quality. Studies suggest that the APP assay may have great potential for the assessment of level of stress and welfare. However, it is necessary first to establish reference ranges for the concentration of these proteins in the normal state, taking into account factors such as sex, age, herd and farm conditions.

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