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Origin and assessment of bruises in beef cattle at slaughter

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Studies of bruises, as detected on carcasses at the slaughterhouse, may provide useful information about the traumatic situations the animals endure during the pre-slaughter period. In this paper, we review scientific data on the prevalence, risk factors and estimation of the age of bruises in beef cattle. Risk factors such as animal characteristics, transport conditions, stocking density, livestock auction and handling of the animals are discussed. Investigation of the age of bruises could provide information on when in the meat chain bruises occur and, could help to pinpoint where preventive measures should be taken, from the stage of collecting the animals on the farm until slaughter. We review the methods available to assess the age of the bruises; data on human forensic research are also included. The feasibility to identify traumatic episodes during the pre-slaughter period, in order to improve animal welfare is discussed.

Keywords: bruise, beef cattle, age of bruises, animal welfare

Introduction

It is generally accepted that the occurrence of bruises has a negative impact on animal welfare as well as on the meat quality of beef cattle.

A bruise – defined as a tissue injury with rupture of the vascular supply and accumulation of blood and serum (Hoffman *et al*., 1998) – develops after the application of force, usually by a blunt object, sufficient to disrupt blood vessels (Bariciak *et al*., 2003). As soon as tissue is damaged, a region of localized hypersensitivity occurs around the injury area. The hypersensitivity of the bruised area minimizes movement of the individual and contact with the injury, until healing has occurred. Thus, it has been inferred that pain is a promoter of repair (Basbaum and Woolf, 1999).

Nowadays, concern for animal welfare is a major consideration in meat production in many countries, and is based on the belief that animals can suffer (Manteca, 1998). Bruising is obviously a source of pain (Gregory, 2004 and 2007). In welfare assessment, pain and the source of pain should be evaluated where possible, in order to establish how far the animal's physical and, also likely, emotional state is affected and that its welfare is poor (Broom, 1986 and 1998).

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Although bruises are inflicted *ante mortem* in cattle, they are not visible in the live animal due to the thickness of bovine skin and can only be detected *post mortem* in the carcasses. It is important to be aware of the possibility of finding *post-mortem* artefacts during the evaluation of bruises. 'Pseudo-bruises' that resemble true bruises – originated by machinery or handling of carcass at the slaughter line – such as hypostasis, congestion of blood or *post-mortem* injuries are artefacts. Artefacts from after death can lead to misinterpretation and require careful interpretation (Vanezis, 2001).

Bruising in cattle is not only an indication of poor welfare, it also causes substantial economic losses (Grandin, 2000), since bruised meat is not suitable for human consumption and must be trimmed off. A carcass that is bruised may be downgraded or even condemned because it is less acceptable to consumers. Moreover, a bruised carcass decomposes rapidly, since bloody meat is an ideal medium for bacterial growth (FAO, 2001), having a shorter shelf-life.

Bruises can occur at any point of the meat chain, due to inappropriate handling of the animal on the farm or at livestock market, during loading, through road transport and unloading at the slaughterhouse, penning and even during stunning procedures (Jarvis *et al*., 1995). Examples of potential bruising events are inappropriate handling, improper use of sticks by handlers, violent impact of the animals against facilities or impact with other animals (Nanni Costa *et al.*, 2006).

Knowledge on the age of a bruise, combined with information on the timing of pre-slaughter events, may facilitate the identification of the risk factors for bruising and thus provide information on where animal welfare is suboptimal. In this paper, we aim to give a state-of-the-art discussion of the factors and circumstances that cause bruises in beef cattle in the pre-slaughter period, and to consider potential methods of age assessment of bruises after slaughter.

Characteristics of bruises

The response of a tissue to a bruise-inducing event depends on the nature of the mechanical force applied and also on the anatomical location where the force is applied (Hamdy *et al.*, 1957b). As a result, bruises may differ in their site, appearance, extension, shape and severity. Anderson and Horder (1979) have suggested that in beef cattle, external factors (i.e. source, transport and handling) may be responsible for the site where bruises are located in the body of the animal, whereas animal factors, such as presence of horns, sex class and temperament, determine the severity of bruising and may cause deeper lesions.

The assessment of bovine bruises during carcass evaluation at the slaughter plant is a retrospective reflection of all harmful situations endured by beef cattle during pre-slaughter time. Several bovine carcass scores have been developed worldwide to be used at slaughterhouses for commercial purposes. All the scoring systems are based on visual appraisal of bruise characteristics, such as extent, site of bruising, colour, appearance and severity, or a combination of the latter.

Extent and site of bruising area

The Australian Carcass Bruises Scoring System (ACBSS), devised by Anderson and Horder (1979) classifies the severity of bruising according to the surface area of the lesion in three groups: 'slight' (S), 'medium' (M) and 'heavy' (H). A lower-case 'd' is used to indicate that the bruising area comprises deeper tissues, creating three new categories: Sd, Md and Hd. A diagram is used to record the site of the bruise where seven areas are distinguished: butt, rump and loin, rib, forequarter, back, hip and pin. All the bruises present, whether on the left or right side of the carcass, are recorded by the same person.

Jarvis *et al.* (1995) used the ACBSS to quantify the occurrence of bruises of cattle from two different sources; they reported that cattle from livestock markets had more bruises than cattle coming directly from the farm. Furthermore, the researchers using this bruise scoring system found differences in the distribution of the bruises over the animal's body. Compared with animals coming directly from farms, beef cattle from markets presented more bruises on the hip (0.33 mean number of bruises per animal *v.* 0.25; $P < 0.05$), butt (0.50 bruises per animal *v.* 0.40; $P < 0.05$) and back (1.13 *v.* 0.83; $P < 0.001$).

Although the ACBSS enables carcass bruising to be recorded reliably and accurately, the records are based on visual appraisal and according to Anderson and Horder (1979) the system is not totally consistent between scorers.

Regarding the location of the bruises, Hamdy *et al.* (1957b) studied the relationship between the force applied to inflict a bruise and the type of tissue involved in the bovine carcass. They observed that the bruises inflicted over the *gluteus*, *triceps*, *biceps* and *trapezius* muscles of the cows were deeper than those inflicted over the lumbodorsal fascia and the *serratus* muscle. It was concluded that the degree of bruising depends on the thickness and density of the affected tissue and its vascularity. No published studies were found on the relationship between the site and the characteristics of bruises in the bovine carcass.

Colour, appearance and severity

The Finnish Meat Research Institute has developed a carcass-bruising evaluation system based on the colour and severity of the trauma (Honkavaara *et al.*, 2003). Three categories are used in this system: 'none', corresponds to a clean, non-bruised surface; 'slight', denotes a reddish area with damage on the surface and 'severe', means the bruise is reddish, deep and bleeding damage can be observed on the surface.

This scoring method may have shortcomings similar to other methods based on visual appraisal; for example, often a bruise is barely apparent on the surface even though it may extend into the underlying tissues.

Deepness and severity

In several South American countries (Argentina, Brazil, Chile and Uruguay), a bruising grading classification is currently used which is based on the severity of the bruise and the tissues affected in the injured area. However, the use of this grading system is only compulsory in Chile (Chile, 1992 and 2002). The system identifies bruises as 'grade 1', when the damaged area comprises only subcutaneous tissues; as 'grade 2', when the lesion affects subcutaneous and muscular tissue and as 'grade 3', severe bruise, when subcutaneous, muscular tissues and even bones are damaged (fractures). In Chile, carcasses presenting bruises of grade 2 must be downgraded to a lower category, and carcasses with bruises of grade 3, to the lowest category of the carcass grading scale.

Gallo *et al.* (1999) evaluated the characteristics of cattle delivered to 22 Chilean slaughterhouses. Their study comprised the analysis of official records of 114 666 bovine carcasses. Bruising was evaluated using the grading classification based on severity of the lesions. The results revealed that 7.7% of the carcasses had grade 1 bruises, 2.1% had grade 2 bruises and only 0.8% had grade 3 bruises. In contrast to the Australian Bruising Score System, which records all the bruises present in the carcass, in the Chilean system if a carcass has multiple bruises, only the most severe bruise is registered.

Shape and pattern of bruises

A standard protocol for recording bruise patterns might assist researchers to link the shape of the bruises to their cause (Grandin, 2000). The cause of bruising can be determined by the pattern of damage on the carcass, for example, if severe damage occurs and a large portion of the carcass is completely bruised, this might indicate that the animal was trampled in the truck. Grandin (2000) points out that deep bruises, but small in extent, are most likely caused by horns. Bruises that consist of parallel red marks are characteristic of those made by sticks (Weeks *et al*., 2002).

Although current bruising-scoring systems in the slaughterhouses are useful for learning about the prevalence of bruises on slaughtered cattle, epidemiological analyses are required to obtain accurate information on risk factors for the occurrence of bruises and the likelihood of presumed causes.

Factors affecting the occurrence of bruises

Many factors have to be considered when attempting to determine the causes of bruises in beef cattle. The following information is restricted to the characteristics of the animal itself, transport conditions, way of handling and methods of animal selling.

Animal factors

Horned v. *hornless animals*. In the 1970s, it was contended that horns might be the major cause of carcass bruising in beef cattle. Meischke *et al*. (1974) found that the mean bruised tissue trimmed from carcasses weighted 1.59 kg for horned as compared to 0.77 kg for hornless cattle. Some years later, it was speculated that removing the tips of the horns could be an effective measure to prevent bruises. Wythes *et al*. (1985) subsequently studied the effect of tipped horns on cattle bruising in Australia. For their study, the animals were classified into three groups: with entire horns, tipped horns and hornless animals. The differences the researchers found between bruising rate in tipped and un-tipped cattle, whether sent for slaughter as separate groups or together, were not statistically significant, but hornless animals had significantly ($P < 0.05$) less bruising than the tipped and horned animals considered as one group. The authors concluded that tipping is not an effective measure to prevent bruising in cattle.

Cattle behaviour. It is known that in bovines, mixing unfamiliar animals results in more agonistic behaviour, which gives rise to great stress (McGlone, 1986). Agonistic behaviour is a conflict situation between two animals and includes butting, attacking and fighting (Blackshaw *et al*., 1987). Butting and mounting among beef cattle can increase the risk of bruising (Warriss, 1990).

Kenny and Tarrant (1987) observed the response of young Friesian bulls to social re-grouping and the use of an overhead-electrified grid to control mounting behaviour. Mounting was the most common behaviour during social re-grouping. The researches found that bruising occurrence was significantly correlated with the number of times an animal performed mounting ($r = 0.56$, $P < 0.01$), was mounted ($r = 0.44$, P<0.05) or was butted ($r = 0.56$, $P < 0.01$). The overhead electric grid was effective to prevent mounting and to decrease bruising.

The relationship between cattle behaviour and its potential to cause bruising was studied in a large saleyard by Blackshaw *et al*. (1987). Butting, attack and fighting were examined separately. The results showed that the neck and the flank of the animals were butted by other animals more often than the hindquarters. The relative frequency of attack and fights did not differ significantly between horned and hornless animals. When the animals were forced to move, they frequently bumped into objects such as fences, sharp corners, half-opened gates which, according to Blackshaw *et al*. (1987), can lead to severe bruising. The damage ratings of behaviours indicate that the problem areas at the saleyard were drafting, weighing and unloading, due to the combination between rough handling and improper facility design.

More recently, German researchers performed a field study including the transport of 580 animals (bulls, cows and heifers) to estimate the impact of facility design on cattle behaviour and meat quality (von Holleben *et al*., 2003). When cattle were not mixed and were driven in small groups they showed calmer behaviour and fell less during loading and unloading, resulting in less bruising. Surprising was the finding that mounting prevention devices may increase bruising if they are set too low, that is at 20 cm above withers or lower.

Age and sex. In the literature, there is some evidence that the level of bruising also varies with the sex and age of the cattle (Yeh *et al*., 1978; Gallo *et al*., 1999). Jarvis *et al*. (1995) quantified the effect of sex class on the occurrence of the carcass bruising of cattle at two commercial slaughterhouses in the United Kingdom. Bruise scores were calculated by multiplying the number of bruises in each size class (little, slight, medium or heavy) by a weighting factor (slight 1, medium 3 and heavy 5) and adding these values. Little bruises (<2 cm) were not considered. The bruise scores were then divided by the number of animals per group, resulting in a mean bruise score per animal. The researchers found that when heifers were completely separated from steers during transport and handling, the mean number of bruises per animal differed significantly between sex classes. Heifers had significantly ($P < 0.001$) more bruises than steers (bruise score 5.40 *v.* 4.00). This finding tallies with data obtained earlier by Yeh *et al*. (1978), who reported that when kept as separate groups, cows bruise significantly more than steers and bulls. Furthermore, only in cows did the amount of bruising (expressed as weight of bruised tissue trimmed) increase with increased duration of journey.

Weeks *et al*. (2002) have pointed out that physical differences in fat cover, skin and thickness of hide between sexes could affect the susceptibility to bruising resulting from impacts of similar force. Moreover, on the basis of the hypothesis that thin animals bruise more easily than fat animals, Grandin (1998) has suggested that cows have more bruises due to their lack of fat cover.

The effect of age on bruising was investigated by Wythes and Shorthose (1991). They found that bruising was greatest in the heaviest animals – the mature and old cows and oldest steers of the group. These results support the earlier findings of Anderson (1973), that older animals have more bruising.

In Chile it was shown that old cattle are more likely to pass through a livestock market before arriving at the slaughterhouse (Strappini *et al*., 2008), so the fact that old animals have more bruising may not only be due to age, but also due to increased handling.

Breed. It has recently been suggested that some differences in the occurrence of bruises can be attributed to breed (Minka and Ayo, 2007). In studies carried out in West Africa, the behavioural activities of cattle during loading and unloading were assessed in three different *Bos indicus* breeds: White Fulani (long horns), Sokoto Gudale (short horns) and Red Bororo (massive horns). The researchers found that animals of the Red Bororo breed had the highest percentage of injuries and the highest score for behavioural activities. They concluded that this may be related to the fact that Red Bororo animals have massive horns and are aggressive by nature. It appears that breed differences can be attributed to differences in behaviour and to being horned or hornless.

Significant differences in carcass bruising between breeds had been reported earlier by Wythes *et al*. (1985), who found that carcasses of Zebu crossbreeds had a greater bruise score compared with British breed animals. However, some years later, the same authors presented new results. Bruising and muscle properties of *Bos taurus* × *Bos indicus* and *Bos taurus* were compared from seven studies. There were no consistent differences between breeds in bruise score. Based on the results of these studies, it was concluded that individual variation in susceptibility to bruising is more important than genotype differences (Wythes *et al*., 1989).

This finding agrees with the suggestion of Fordyce *et al*. (1985), that differences between individual animals in susceptibility to bruising and in temperament might be more important than the variability between breeds.

Transport

Distance, time and transport conditions. Road transportation can be associated with several types of injuries (Minka and Ayo, 2007). Many authors have emphasized the relation between distance travelled and occurrence of bruising in bovines (Yeh *et al*., 1978; McNally and Warriss, 1996; Hoffman *et al*., 1998), suggesting that the level of bruising might increase with the distance travelled by the animals and consequently the amount (kg) of bruised tissue trimmed per carcass (Wythes *et al*., 1981). However, Tarrant and Grandin (2000) postulated that the condition under which the transport takes place is more important than the total journey time or the distance covered. After the animal has adapted to the situation, time is a minor problem compared to loading densities, vehicle design, road conditions or the driver's driving behaviour. Previously, Tarrant *et al*. (1992) found that 600 kg cattle began to lie down after 16 h of transport, but at the highest stocking density of 600 kg/m^2, the animals could not rest because of the lack of space. Although cattle prefer to stand during transport, they do lie down during long journeys (Knowles, 1999). Thus, preventing animals from resting after 16 h or more of transport may become an important animal welfare issue in many countries.

Studies of the relationship between vehicle design, transit conditions, climatic conditions, transport time and distance are required to get a better insight about their effect on bruising occurrence.

Stocking density. It has been speculated that the extent of bruising increases with increased stocking density during transport.

Tarrant *et al*. (1988) transported cattle at three different stocking densities: low (200 kg/m^2), medium (300 kg/m^2) and high (600 kg/m^2). Carcass bruising was scored using the ACBSS. The bruising scores were 3.1 at 200 kg/m^2, 3.6 at 300 kg/m^2 and 11.9 at 600 kg/m^2, respectively. From these results, it was concluded that carcass bruising increases with increased stocking density.

Cattle transported at high stocking density have limited room to move and to adopt preferred orientations, such as to align themselves with the direction of the travel, which may increase their security of balance. An interesting observation at high loading density was the 'domino effect', whereby a fallen animal caused others to lose their footing. Trampling on the floored animal destabilized other members of the group and this resulted in more animals going down. It is likely, that occurrence of the 'domino effect' is related to the driving style, because the majority of incidents in which cattle adjust their position, stumble or fall are associated with sudden changes such as braking, gear changes or cornering (Knowles, 1999).

Not only overloading, but also under-loading of trucks increases bruises. Eldridge and Winfield (1988) transported animals at three different stocking densities: high (460 kg/m^2), medium (345 kg/m^2) and low (288 kg/m^2). The Australian researchers found that carcass bruising was higher in both the high and low stocking density treatments compared with the medium treatment.

The contradiction between the findings of Tarrant *et al*. (1988) and those of Eldridge and Winfield (1988), in relation to adverse low stocking densities, may be explained by the differences in average live weight of the animals (603 and 400 kg, respectively) used in these experiments. In any way, it is clear that at low stocking densities, loose animals try to keep their balance in a moving truck and are more likely to hit the vehicle's walls and tailgate.

It seems that a solution could be to transport animals in pens. Honkavaara *et al*. (2003) carried out several experiments in Finland using vehicles in which there were large pens (three or four animals per pen) or small pens (one or two animals per pen). The authors showed that two- and single-animal pens were optimal to minimize aggressive behaviour and carcass bruising during transport, presenting an alternative for transporting animals – especially over long distances. Unfortunately, the use of movable barriers is not a common practice in most South American countries where cattle are transported loose in one compartment, at high stocking densities (Grandin and Gallo, 2007).

The relationship between stocking densities and bruising incidence requires further research in order to provide policy makers with scientific information that can be used to define national regulations appropriate to the local situation.

Livestock markets, slaughterhouses and handling

In most countries, a high percentage of beef cattle are still marketed through live auction markets, a process which extends transport times and multiplies the number of occasions that animals are loaded, unloaded, driven and mixed with unfamiliar animals (Knowles, 1999). All of these conditions are associated with the risk of physical damage and bruising.

Blackshaw *et al*. (1987) performed behavioural observations on about 2400 cattle throughout the livestock market routine in Australia. It was observed that animals showed agonistic behaviours during drafting, weighing and unloading stages, which involve stock handlers moving animals. McNally and Warriss (1996) found that the prevalence of bruising was significantly higher in animals bought from live auction markets (7.8%) than in those bought through dealers (6.3%) or direct from farms (4.8%), suggesting that when animals are handled more, they are exposed to more potentially traumatic situations.

Weeks *et al*. (2002) attempted to identify potential bruising events caused by handling at livestock markets. They also found that animals that had passed through a market presented more bruises (71.0% of carcasses, $n = 1.095$) than cattle delivered by dealers (65.5%, $n = 1.925$) or from farms (53.7%, $n = 1.980$). It was concluded that the more an animal is handled, the greater the chance of developing bruises.

However, other studies indicated that animals sold through livestock markets did not present more bruises than cattle sold directly to the abattoirs (Horder *et al*., 1982).

Cattle transported direct from the farms to the slaughterhouse may be less tired or may find the lairage environment less familiar than the market cattle (Jarvis *et al*., 1995). According to Grandin (1993), if the animals are not tired, handling can be more difficult, especially if the animals are excited and therefore subjected to rough and abusive handling. This corresponds with the finding of Jarvis *et al*. (1995), who found significantly greater use of driving instruments on cattle transported directly from farms than on animals sold through markets.

Based on the existing evidence, it has been concluded generally that animals subjected to additional handling and transport associated with livestock market processes will present more bruising (Jarvis *et al*., 1995).

An earlier survey conducted by Marshall (1977) in New Zealand, reported that bruising was directly related to the method of handling of cattle. Lensink *et al*. (2001) investigated the influence of farmers' handling of veal calves during loading, transport and unloading. The authors found that animals receiving positive contact from the stockperson are less fearful of people, resulting in fewer potentially traumatic incidents. Unfortunately, many stockpersons are not trained to handle animals in a proper way (Grandin, 1980).

Cattle can be bruised up until the moment of processing, furthermore, bruising can occur after stunning and prior to bleeding (Meischke and Horder, 1976). In relation to the latter, McCausland and Millar (1982) found that at least 43% of the bruising occurred after the animals arrived at the Australian slaughterhouses. Nevertheless, it is commonly assumed that bruises are inflicted before arriving at the slaughterhouse, because the probability of developing bruises in the slaughterhouse is rarely considered. Given that market cattle have an increased risk of becoming bruised during transport from and to markets, on arrival at the slaughterhouse the bruises will be old. But cattle transported directly from farms have a higher risk to present fresh bruises because of more handling problems at the slaughterhouse itself. Therefore, depending on the severity of abuse during loading and transport or at the slaughterhouse, the comparisons in literature between market cattle and farm cattle may differ.

It is clear that the way of handling, the use of driving instruments and the level of exhaustion affect the risk of bruising in animals passing through markets. More research should be done on the age of bruises found on carcasses, in order to elucidate the link between bruise occurrence and livestock auction and slaughterhouses, so as to pinpoint where adverse handling has occurred during the period from loading to slaughterhouse.

Estimating bruise age

In the 1950s, Hamdy and co-workers collected evidence of biochemical and physical changes in bruised tissues, indicating that the estimation of the age of a bruise allows the identification of the place and time of livestock damage and provides information about the causes (Hamdy *et al*., 1957a and 1957b). Since then, different methods have been employed to estimate the age of bruises in animals.

Bruise colour changes

Gracey and Collins (1992) showed that the age of the bruise can be estimated from its colour appearance in bovine carcasses; a bright red bruise is likely to be up to 10 h old, whereas a dark red bruise is approximately 24 h old. This change in bruise colour is due to the inflammatory process, whereby macrophages are recruited to the injured area and ingest red blood cells and metabolize the haemoglobin first to biliverdin and then rapidly to bilirubin (Hughes *et al.*, 2004). Based on empirical observations, Grandin (2000) concluded that in beef cattle carcasses it would be possible to separate bruises into at least two categories: fresh bruises and bruises that are several days or weeks old. The latter would be indicated by the presence of yellow colour in the damage area, attributed to bilirubin levels.

Northcutt *et al.* (2000) assessed the age of bruises in broilers, based on colour measurements. They reported a colour transition: initially red and then continuing through shades of purple, green and yellow. Broiler bruises appeared green after 24 h. Nevertheless, the researchers found that bruise appearance in broilers was affected by location, with breast bruises becoming darker with increasing bruise age, whereas wing and drum bruises becoming lighter. Northcutt and co-workers explained that this variation in colour was caused by the veins in the wing being situated close to the skin surface.

From extensive studies of different species (Langlois and Gresham, 1991; Langlois, 2007), it was concluded that only the appearance of a yellow colour may provide information on the age of a bruise, recommending that no attempt should be made to analyse other colours such as blue, green, purple, black, orange, brown or red, because a bruise may contain different colours at any one time (Maguire *et al.*, 2005). Langlois (2007) stated that if yellow colour is seen in a bruise, the bruise is not recent and should be aged as older than 18 h. Nevertheless, it has not been accurately established when yellow colour appears in a bruise and this may also differ between species.

In their research, Hughes *et al.* (2006) found that there is wide variation in the threshold for the perception of yellow colour between observers. Methods based on visual colour changes have low reliability and accuracy for estimating bruise age.

Chemical test

Hamdy *et al.* (1957a) developed a chemical test based on bilirubin and biliverdin levels to determine the age of bruises in cattle and rabbits. It was concluded that the test failed to detect bilirubin in the early stages of healing, due to the slow degradation of haemoglobin. The bilirubin tissue analysis does not accurately establish the age of the trauma if the bruises originate 50 h or less before slaughtering. This makes this method less suitable for investigating pre-slaughter transport events.

Histological studies

McCausland and Dougherty (1978) used microscopic examination in bruise cell populations in cattle. Fresh bruises contained few neutrophils and macrophages. Eight-hour bruises contained extensive tissue haemorrhage, fragmented muscle fibres, numerous neutrophils, but few macrophages. Bruises which were 24 h old had neutrophils and macrophages closely associated with damaged fibres. A few years later, McCausland and Millar (1982) applied the same histological ageing method to cattle at two abattoirs in Australia. Prussian blue was used to detect haemosiderin. The age of each bruise was related to the time of arrival of the animal at the slaughterhouse, where 0 h corresponded to a bruise sustained at slaughter. The results showed that most of the bruises were categorized as having occurred at the slaughter (0 h), apparently occurring in the hours before or after stunning. The method was not sensitive enough to accurately estimate the age of a bruise.

Using a Bayesian probability model, Thornton and Jolly (1986) evaluated histological data of bruises inflicted on sheep at different times. The model was developed using data from one tissue section from 20 bruises and then tested using data from the remaining tissue section. Using this model, it was possible only to age bruises with 90% of confidence as 1 to 20 h old or 24 to 72 h old.

To conclude, histological methods are simple to apply, but they can only discriminate between old bruises (more than 24 h) and fresh bruises (less than 24 h). More accurate methods are needed to estimate the age of a bruise in the immediate period after infliction in terms of minutes to hours.

Enzyme histochemical methods

These methods are based on the determination of the presence and changes of the enzyme reaction in the bruised area. Raekallio (1965) reported a key finding, showing that it is possible to detect and localize enzymatic activity such as esterases, β-glucuronidase, adenosine triphosphatase and monoamine oxidase, in the earliest period of healing, proving that this is not an inert period. However, enzymatic activity inside the bruise itself varies and it is possible to clearly discern two zones: the central zone located up to 500 μm from the bruise edge and the peripheral zone, a portion up to 100 to 200 μm from the central zone. The enzymatic activity decreased at the central zone over time, and this change was detected 1 to 4 h after bruising. In contrast, in the peripheral zone, enzymatic activity increased over time and was detected 1 h after the bruise was sustained.

More recently, Psaroudakis *et al.* (2001) used rabbits to investigate the enzymatic activity in bruises. The results showed increased activity of nonspecific esterases approximately 1 h old, followed by an increase in adenosine triphosphatase at approximately 2 h and alkaline phosphatase at approximately 3.5 h. Peak enzyme activity for nonspecific esterases occurred 24 h after wounding in rabbits, compared with 20 h for adenosine triphosphatase and 32 h for alkaline phosphatase. The researchers affirmed that the enzyme histochemical methods used are simple, inexpensive and give reliable and reproducible results after a minimum of 1 h after bruising.

However, Grellner and Madea (2007) questioned the enzyme histochemical methods, arguing that they are too unreliable and show a high rate of negative cases, even after periods of several hours. Despite the negative results of Grellner and Madea, it would be worthwhile to carry out more systematic investigations of the use of enzyme histochemical methods to age bruises in bovine carcasses.

Forensic investigation of human skin bruises

Establishing the time a bruise was incurred has considerable importance in human forensic pathology research, especially in relation to victims in child abuse cases (Sawaguchi *et al.*, 2000). The latter accounts for the numerous studies carried out in recent decades with the aim of developing a method for ageing bruises in human skin (Langlois and Gresham, 1991; Betz, 1994; Sawaguchi *et al.*, 2000; Bariciak *et al.*, 2003; Bonelli *et al.*, 2003; Hughes *et al.*, 2004 and 2006; Randeberg *et al.*, 2006; Grellner and Madea, 2007; Kondo, 2007).

The most common techniques used by practitioners to estimate the age of human skin bruises are either direct visual evaluation or inspection of photos (Langlois and Gresham, 1991). These methods are subjective, rely on experience and individual visual perception, and depend on ambient lighting and photographic quality (Randeberg *et al.*, 2006). Moreover, the appearance of a bruise in the human skin is influenced by its location, the individual's tendency to bleed, skin colour, and the force of injury, depth and extent of subcutaneous extravasations (Maguire *et al.*, 2005). These methods are neither accurate nor reliable.

Regarding objective methods used in forensic investigation, it has been found that reflection spectroscopy was a valuable method to monitor skin reactions following non-penetrating trauma (Randeberg *et al.*, 2006). However, deep muscular haemorrhages could not be detected at an early stage.

Nowadays, immunohistochemical, biochemical tests and molecular biological techniques are mainly used to study the age of human skin bruises in forensic medicine. Some are summarized below.

Bonelli *et al.* (2003) demonstrated that the density of mast cells (MCs) is significantly higher in bruises sustained *ante mortem* than in healthy skin or in *post-mortem* lesions. Histamine content in bruises increases with time, peaking after 3 h, and falling to a minimum 24 h after bruising. Since the main source of skin histamine are MCs, the distribution and number of these cells might be used for establishing bruise age. The researchers stated that the technique can be performed on routinely fixed and stored tissue samples and does not require dedicated procedures. The cytochemical analysis of MCs can be combined with other morphological analyses on the same tissue block, as the reagents are relatively cheap and the procedure can be performed in any forensic pathology laboratory.

According to Betz *et al.* (1992), fibronectin, a multifunctional cell adhesion protein, is probably the most sensitive marker for determining bruise age. Evidence supporting this, is that some bruises, 10 to 20 min old, showed an immunopositive reaction to fibronectin (Betz *et al.*, 1992).

In recent years, adhesion molecules have been identified, revealing a cascade of bonding reactions. The adhesion molecules intervene in the interaction between leucocytes and endothelial cells during the inflammatory phase of skin healing. Dressler *et al.* (2000) found a strong immunopositive reaction to P-selectin at the earliest 3 min after injury and at the latest after 7 h. The expression of E-selectin, another adhesion molecule, was evident in 1-h-old bruises. The immunohistochemical detection of adhesion molecules does not make excessive demands on laboratories (Dressler *et al.*, 2000).

Cytokines are multifunctional glycoproteins which are closely involved in various biological events. Interleukin (IL)-1, IL-6, and tumour necrosis factor (TNF)-α are representative pro-inflammatory cytokines (Kondo, 2007); several experiments demonstrated that these pro-inflammatory cytokines were up-regulated at both protein and mRNA levels at the injury site, suggesting that they could become markers for bruise age determination.

Also involved in wound healing are transforming growth factors (TGF) (Grellner *et al.*, 2005). The semi-quantitative evaluation of immunostaining intensity for TGF-α and TGF-β1, revealed that their expression was enhanced within the first hour after bruising, suggesting that they could be useful markers for bruise age determination, particularly as they are easy to evaluate.

The crucial issue in bruising age investigation is to find an accurate, reliable and feasible usable method, whether the interval between the bruising incident and the *post-mortem* evaluation to estimate the age of bruises is minutes or hours. The immunohistochemical detection of cytokines, adhesion molecules, collagens and growth factors seem to be a promising techniques for this (Grellner, 2002; Grellner and Madea, 2007; Kondo, 2007).

Despite the fact that adhesion molecules and cytokines may be identified in bruises, it is not clear how their concentrations change over time and thus allow age determination of bruises. Moreover, if these concentrations are assessed by immunohistochemistry alone, they imply a substantial degree of subjectivity in determining results. Biochemical analysis of the specific concentrations of these proteins would be far more reliable but may entail complex procedures.

Amplification of their corresponding mRNAs by real-time PCR would be another possible method, but even this is not strictly a quantitative technique.

Discussion

The literature provides clear evidence of a number of external causes of bruises that are sustained during the last hours and days before the animals are slaughtered. Animal factors such as sex and age may contribute to the development of bruises, at least in some cases. Better understanding is still needed of the biological mechanisms accounting for the higher bruise rates in females and older animals. It is clear that beef cattle sold through markets can suffer bruising that could have been avoided by transporting animals directly from the farm to the slaughterhouse.

Many aspects of cattle transport contribute to bruising. Transport conditions, such as stocking density and duration of the journey seem to have more effect on bruising than distance travelled. However, finding an optimal stocking density for livestock transport under different conditions is still a contentious issue.

Bruised tissues may store historical information about the harmful situations that the animal underwent prior to slaughter. The farmer and the transport companies have economic incentives to prevent and reduce bruising. However, slaughterhouses do not have simple and accurate methods for *post-mortem* age estimation of bruises to assess accurately when bruises were sustained. This is a relevant problem, due to the importance of having to decide who is economically accountable for the losses. Although the number of bruises, their anatomical location, severity and even the healing process might offer a rapid tool for identifying and evaluating the circumstances during the pre-slaughter period such as high stocking density, rough handling or inappropriate facility infrastructure, other sensitive techniques should be considered for refined assessments of the time the bruises were incurred.

The risk conditions leading to bruises differ in duration from minutes to hours, and even days. Transport and lairage are likely to be the conditions lasting the longest: from half an hour to 2 days or more. In contrast, loading, unloading or stunning procedures may last only minutes. As a result, more sensitive methods are required to detect the earliest point in time at which bruising occurs.

Clearly, more investigation of the time between bruising and slaughter may help to elucidate the risk factors that have contributed to the occurrence of bruises and thus will also help identify the risks for animal welfare.

The modern diagnostic techniques applied when evaluating human bruises, may be studied for bovine bruises as well. Immunohistochemistry and cytochemistry seem to be promising methods to be applied to measure morphological or biochemical changes which can clearly be distinguished from non-bruised tissues. However, age assessment of bruises continues to be a crude process. A wide variety of factors intrinsic to the animal can influence the inflammatory process and subsequent repair. Normal biological variation among animals is therefore bound to result in substantial overlap among proposed time frames in the healing process.

The existing data are sufficient to indicate *a priori* that assessing the age of bovine bruises, might be a helpful tool to identify traumatic episodes during the pre-slaughter period, in order to improve animal welfare; they also have relevance for meat quality assessment.

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