Bruises in Chilean cattle:

Their characterization, occurrence and relation with pre-slaughter conditions

Ana Carolina Strappini

Thesis committee

Thesis supervisors
Prof. dr. ir. B. Kemp
Professor of Adaptation Physiology
Wageningen University

Prof. dr. ir. J.H.M. Metz
Emeritus professor of Farm Technology
Wageningen University

Thesis co-supervisors
Dr. ir. K. Frankena
Associate professor, Quantitative Veterinary Epidemiology Group
Wageningen University

Dr. C.B. Gallo
Senior lecturer, Animal Science Institute
Universidad Austral de Chile

Other members
Prof. dr. H.A.P. Urlings, Wageningen University; VION Food, Eindhoven, The Netherlands
Prof. dr. ir. M.W.A. Verstegen, Wageningen University
Prof. dr. M. Naguib, Wageningen University
Dr. E. Lambooij, Wageningen UR Livestock Research, Lelystad

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Thesis

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Abstract

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Bruises on cattle carcass affect the quality of the meat and are indicators of poor welfare conditions. According to the literature the occurrence of bruises is related to pre-slaughter conditions, however their contribution is not clear for Chilean cattle. The aim of this thesis was to provide a better understanding of the relationship between pre-slaughter factors and the occurrence of bruises -from loading until slaughter- under Chilean conditions. Therefore in the first study slaughter records of two Chilean slaughterhouses were analysed. It showed that cows and oxen had higher risk to present bruises compared to steers and heifers. Moreover, animals that passed through a livestock market were more prone to present bruises than animals that came directly from the farm. A large difference in carcass bruise prevalence was found between slaughterhouses and this discrepancy was attributed to differences in the use of the Chilean scoring system and to several constraints of the system itself. Thus a new scoring system was developed and its reliability was assessed showing a high agreement when only one observer performs the scoring. An inventory of the gross characteristics of bruises, based on the refined bruising protocol, was carried out. Animals passing through a livestock market have more bruises than animals transported directly from the farm to the slaughterhouse. This thesis presents evidences of rough handling and animals beaten by sticks at markets. In the last study the causal event of bruises during the pre-slaughter period was assessed. It showed that rough handling due to inappropriate use of aids to drive animals during loading and unloading, and inadequate stunning facilities at the slaughterhouse were the areas of most risk for bruising. It was concluded that improvements in the design and maintenance of appropriate structures and training of stock people will reduce the occurrence of bruises and in consequence will lead to better welfare conditions of cattle for slaughter.

In memory of my dear parents,

who instilled the love and respect for animals in me…

Contents

Chapter 1

General Introduction

1. Introduction

1.1 The Chilean meat industry and the issue of carcass bruises

Chile aims to become a leading world food producer and to achieve this goal the government is implementing the 'food power program'. This program seeks to develop the food export sector and to monitor all aspects of the production chain (Foreign Investment Committee, 2010). The improvement of the meat industry is part of the program. The need for change was prompted in 2002 when a Bilateral Free Trade Agreement (FTA, 2002/979/EC) was signed between Chile and the European Union. As a consequence, the Chilean red-meat industry has gained access to new markets and export has grown steadily. Despite the technological advancements that were achieved after the agreement, large losses in the form of carcass condemnation and meat trimmed due to bruising continued (Gallo, 2009) (Figure 1).

Figure 1. Cattle carcass with bruises (left) and without bruises (right)

Severely bruised carcasses –in which muscle or bone are affected- cause economic losses to the meat industry because bruised tissue must be trimmed and by that, the quantity of the meat on the carcass will be reduced (INN Chile, 2002). Vidal et al (2007) estimated that in 2006 the red-meat industry lost 1.17 million US$ due to bruised carcasses, considering that around 950,000 animals were slaughtered.

Nowadays, there is an increased public concern about animal welfare by the consumers of export markets. European consumers care about the way meat is produced and marketed (Eurobarometer, 2007). In contrast, Chilean consumers are more concerned about the price of the meat and put less emphasis on the welfare of the animals before slaughter (Schnettler et al., 2008). Since carcass bruises are indicators of sub-optimal welfare conditions during production and pre-slaughter practices, bruised carcasses cannot be exported and therefore they have to be commercialized within domestic markets at lower prices.

Bruises are traumatic lesions (Gracey and Collins, 1999) that develop after the application of force. Some methods commonly used to drive cattle, such as hitting or poking with a sharp or blunt object, produce not only physical injuries and pain to the animal (Gregory, 2004) but also aversive emotional states and fear (Grandin, 2002). Frequently, these practices coincide with shouting by the handlers, tail twisting or shocking with electric prods (de Vries, 2011) which may negatively affect the welfare of the animals as well.

Due to the relationship of bruises with animal welfare, its impact on meat quality and economic losses as a consequence, the Chilean Foundation for Agricultural Innovation (FIA) together with the Chilean Association of Meat Plants (FAENACAR), in collaboration with the Universidad Austral de Chile (UACh) carried out the project "Diagnosis of the situation and development of strategies to improve animal welfare and meat quality in ruminants for human consumption" (FIA-PI- C- 2005-1). The project -running between 2005 and 2009- aimed to quantify the level of bruising and to identify adequate solutions. This thesis is part of that project.

2. The occurrence of bruises under Chilean conditions

The Southern part of Chile is one of the rainiest areas of the world, rich in grazing land. In this area –mainly in the Bío-Bío, La Araucanía, Los Ríos and Los Lagos Regions- the majority of the Chilean cattle population(dairy and beef type) is located (64.2% of the total of 3.6 million of heads) and 44% of the Chilean red-meat is produced (of the total of 210,000 tonnes) (ODEPA, 2010). Beef cattle production is closely related to the dairy sector as the same stock is often used to supply calves for both milk and meat production. Cattle slaughtered for human consumption belong to Holstein, red and black Friesian or half-blood, crossed with Hereford and Angus breeds. The main category processed for meat is steers (49.2%), followed by cows (25.4%), heifers (18.2%), oxen (3.2%), bulls and bullocks (2.8%), and calves (1.0%) (ODEPA, 2010).

Large differences in bruise prevalence appear from the results of several studies, varying from 7.7% (Gallo et al., 1999) up to 64.1% (Matic, 1997). Part of the variation may originate from the use of the bruise scoring system. Gallo et al. (1999) used the database of the bruise scoring made by the official meat graders (INN Chile, 2002) which focuses more on severe bruises affecting deepest tissues, whereas Matic (1997) estimated the prevalence by directly observing and registering all bruises present on the carcass, regardless their severity.

A number of factors appear to contribute to the presence of bruises in Chilean cattle e.g. long lasting transport under high stocking densities (Gallo et al., 2005; Tadich et al., 2005), trade of the animals through livestock auction markets (de Vries, 2011), long lairage time at slaughterhouses under fasting conditions (Gallo et al., 2003), lack of training of stockpersons (Strappini et al., 2006), rough animal handling, and some characteristics of the Chilean cattle, such as presence of horns (Gallo, 2009).

The transport of cattle is regulated by Law N° 19.162 (Chile MINAGRI, 1992) which defines the characteristics of trucks and the allowed maximum stocking density. In 2006, as part of the project FIA-PI- C- 2005-1, an assessment to evaluate the compliance with this regulation was carried out (Strappini et al., 2006). The results showed that most of the trucks comply with the requirements related to the equipment (flooring, height of walls, presence of a sign that identifies it as a livestock-truck, etc.). However, several events that might lead to bruising were observed at arrival at the slaughterhouse. When only one leaf of the rear door was opened, most of the animals impacted with the closed part of the door during unloading. The probability of injuries increased further when animals are forced with violence to move by the use of sticks and electric prods or kicking by a person. Another potential cause of body damage is the difference in height between the truck and the unloading ramp or platform. When the platform is significantly lower than the truck, animals were observed falling and slipping during unloading. Average stocking density observed at arrival at the slaughterhouses was 461±49 kg/m^2 (Strappini-Asteggiano, 2010) which is within legal limits since the maximum stocking density in Chile is 500 kg/m^2. However, this density largely exceeds the allowed maximum in the European Union (360 kg/m^2).

Bruises with a tram-line appearance are frequently observed on carcasses of animals coming from livestock auction markets (Gallo, 2009). This characteristic type of bruise is caused by a round shaped object (Weeks et al., 2002) or by the application of an electric prod (Gallo, 2009). A recent study carried out in Southern Chile, evaluated the quality of the human-animal relationship (HAR) at 11 livestock markets, and associated it with the stockpersons knowledge and attitude (de Vries, 2011). Although most of the stockpersons

knew the effect of hitting (83.3%) on carcass bruising and animal welfare, at livestock markets, sticks are the most used tool to drive animals from one place to another (Figure 2).

The design of the livestock market also contributed to the risk of bruising. De Vries (2011) found that narrow and dark entrances can induce hitting by the stockpersons because cattle naturally tend to move from a darker area to a more brightly lighted zone (Grandin, 2007). Furthermore, working under pressure in a busy environment and the negative attitudes towards animals of the colleagues of the stockperson were also factors that promoted the use of the stick. Since stockpersons are not formally trained, most attitudes probably develop from observations of other stockpersons and personal experience of their own interactions with farm animals (Hemsworth, 2003).

Figure 2. Stock person moving animals with a stick at a Chilean livestock market

A higher proportion of bruises was reported in cows and oxen compared to younger categories like heifers and steers (Godoy et al., 1986; Gallo et al., 1995; Gallo et al., 1999) and can be associated with the poor body condition of the older animals (Grandin, 2002).

When the causes of the bruises are known improvements can be reached. However, from the current *post mortem* carcass bruising evaluation, it is not possible to assess accurately 'where', 'when' and 'how' bruises were inflicted. This latter is a relevant issue for the Chilean meat sector, due to the importance of having to decide 'who' is economically

accountable for the losses in one hand, and to identify the factors which impaired the ethical quality of the meat on the other.

3. Objective of the thesis

This thesis aims to study the relationship between pre-slaughter factors and the occurrence of bruises (from loading until slaughter) under Chilean conditions, and is structured as follows.

Chapter 2 provides a literature review presenting a state of art on factors that contribute to the occurrence of bruises in slaughtered cattle. Studies from various countries are summarized, with respect to the role of animal characteristics, transport conditions, stocking density, livestock auction, lairage conditions and handling of the animals. In **Chapter 3**, bruise prevalence in Chilean beef cattle is assessed and risk factors are studied based on slaughter records of over 130.000 carcasses and using the Chilean bruise scoring standard used by the meat graders (INN Chile 2002). The Chilean bruise scoring system is quite global and only gives information related to the most severe bruise in terms of depth of the infliction. No information about other bruises on the carcass, site of bruise, size of the bruise, shape and colour is provided. More detailed data on each bruise would allow a better deduction of potential bruising events and the pre-slaughter welfare circumstances for the animal. Therefore a new and more detailed scoring system was developed. The intra and inter-observer reliability of this bruise scoring system is described in **Chapter 4**. In **Chapter 5** an inventory of the gross characteristics of bruises, based on the refined bruising protocol, was performed where cows coming from livestock market or directly from farms were compared. **Chapter 6** describes a study about 'when', 'where' and 'how' potential bruising events take place during transport from farm to slaughter. Furthermore, the potential bruising events are related to the *post mortem* visual appearance of the bruises. Recommendations to prevent bruises under Chilean conditions and final remarks related to the impact of bruises to the welfare of slaughter cattle conclude the thesis (**Chapter 7**, General Discussion).

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Chapter 2

Origin and assessment of bruises in beef cattle at slaughter

A.C. Strappini [1,2], J.H.M Metz [3], C. Gallo [2] and B. Kemp [1]

[1] *Adaptation Physiology Group, Department of Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH, Wageningen, The Netherlands*
[2] *Instituto de Ciencia Animal, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Casilla 567, Isla Teja, Valdivia, Chile*
[3] *Farm Technology Group, Department of Agrotechnology & Food Sciences, Wageningen University, P.O. Box 17, 6700 AA, Wageningen, The Netherlands*

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Abstract

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).

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 artifacts 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 artifacts. Artifacts 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 MINAGRI 1992; INN Chile, 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 vs. 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 50.56, $P < 0.01$), was mounted (r 50.44, $P < 0.05$) or was butted (r 50.56, $P < 0.01$). 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 x 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/m2, 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/m2), medium (300 kg/m2) and high (600 kg/m2). Carcass bruising was scored using the ACBSS. The bruising scores were 3.1 at 200 kg/m2, 3.6 at 300 kg/m2 and 11.9 at 600 kg/m2, 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/m2), medium (345 kg/m2) and low (288 kg/m2). 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 conside1red. 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. Eighthour 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 usingdata 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, b-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 mm from the bruise edge and the peripheral zone, a portion up to 100 to 200 mm 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 1h 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)-a 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-a and TGFb1, 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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Chapter 3

Prevalence and risk factors for bruises in Chilean bovine carcasses

A.C. Strappini [a,b], K. Frankena [c], J.H.M. Metz [d], C. Gallo [b] and B. Kemp [a]

[a] *Adaptation Physiology Group, Wageningen University, P.O. Box 338, 6700 AH, Wageningen, The Netherlands*
[b] *Institute of Animal Science, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Casilla 567, Isla Teja, Valdivia, Chile*
[c] *Quantitative Veterinary Epidemiology Group, Wageningen University, P.O. Box 338, 6700 AH, Wageningen, The Netherlands*
[d] *Department of Agrotechnology & Food Sciences, Farm Technology Group, Wageningen University, P.O. Box 17, 6700 AA, Wageningen, The Netherlands*

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Abstract

Records of cattle slaughtered at two Chilean slaughterhouses (SLH1 and SLH2) were used to determine prevalence and risk factors for carcasses with bruises. Bruise prevalence amounted to 12.3% but differed between slaughterhouses (20.8% for SLH1 and 8.6% for SLH2 respectively). Bruise severity grade 1 (mild) was most frequently recorded. The type of the animal, source of animal, the level of fat cover and lairage time were associated with the presence of bruises. Older categories of animals and animals that pass through a market before being moved to the slaughterhouse are more prone to show bruises The results also indicate that under the reported Chilean circumstances animals that have longer lairage times (over 12 hours) have a significantly reduced risk for bruises, except for oxen. Presence of bruises is also significantly associated with increased carcass pH values.

Keywords: *bruises, bovine carcass, risk factors*

1. Introduction

Cattle are exposed to various handling and transport conditions during marketing and previous to slaughter. When these conditions are associated with rough handling, violent impact of the animals against protuberant or sharp-edged surfaces, or with aggression between animals, mechanical damage to the animal tissues can occur which may develop into a bruise. A bruise or "contusion" is a traumatic injury with rupture of the vascular supply and accumulation of blood and serum in the affected tissue (Gracey et al., 1999).

Handling and transport conditions may vary according to the way of selling the animals. Some cattle are sold and shipped directly from the farm to the meat plant minimizing the effect of (un)loading procedures. Others are sold through private dealers who have their own facilities for handling and transport of the animals from farm or market to the slaughterhouse. Also animals are sold through live auction markets, resulting in longer transport times, multiple (un) loading procedures, and likely mixing with unfamiliar animals (Knowles, 1999). Carcasses of cattle arriving at the slaughterhouses from markets are reported to have a significantly higher prevalence of bruising than those arriving directly from the farm or sold through a livestock dealer (McNally and Warriss, 1996; Weeks et al., 2002).

Also animal factors such as sex, age, type of animal and fat cover were found to affect the development of bruises in cattle (Jarvis et al., 1995; Voisinet et al., 1997). In the literature there is evidence that female animals have higher chances for bruises than males (Yeh et al., 1978; Jarvis et al., 1995) and similar results were observed for older animals compared to young stock (Wythes and Shorthose, 1991; Anderson, 1973). Differences in fat deposition and subcutaneous tissue organization between cattle of different sex and age (Eguinoa et al., 2003) might explain the higher bruise susceptibility in female and older animals.

In relation to fat cover, some authors hypothesized that thin animals would bruise more easily than fat animals due to a lack of fat cover (Knowles et al., 1994; Jago et al., 1996), but the relationship has not been studied into detail.

An association between lairage time and presence of bruises was reported for cattle and other species (in cattle, Warriss,1990; in sheep Cockram and Lee, 1991a; in pigs, Fraqueza et al., 1998; in rabbits, Liste et al., 2009; and in deers, Jago et al., 1996). All the reports indicate an increased number of bruises when the lairage time increases.

Seasonal factors -mainly temperature and humidity- also seem to play a role in the prevalence of bruises. Eldridge and Winfield (1988) found that cold weather combined with

precipitation increased the mean number of bruises per animal, although the reasons are still unclear.

Prevention of bruises is important because bruises are indicative of violence and pain that animals underwent, referring thereby to poor welfare conditions during pre-slaughter period (Strappini et al., 2009). Moreover, bruises in bovine carcass affect the quality of the carcass and the meat. In Chile, bruised carcasses are downgraded and the bruised tissue must be trimmed reducing the economic value of the carcass (Gallo, 2008). Another reason for downgrading carcasses is the presence of dark, firm and dry (DFD) meat. The relation between bruises and DFD meat in bovine carcasses has not yet been studied at large, but is relevant to consider.

In Chile, a country that aims to improve beef production and meat quality in a short term, epidemiological information regarding intensity, spread and risk factors of bruises is limited. Gallo et al. (1999) evaluated the prevalence of bruising in cattle slaughtered at 22 Chilean slaughterhouses (n= 114,666). The results revealed that 7.7% of the carcasses had bruises; in 4.8% of the carcasses only subcutaneous tissues were affected, in 2.1% muscles were affected and 0.8% of the carcasses presented bruised areas affecting both subcutaneous, muscle and bone tissues. However, risk factors related to carcass bruises have not been studied yet. The present study aims to assess the prevalence of carcass bruising based on data of two Chilean abattoirs over a one year period of time and to quantify its relation with animal characteristics, season conditions, the method of selling and lairage time. The association between presence of bruises and carcass pH was assessed, pH being a proxy for meat quality, specifically DFD meat. The study was based on data recorded by two slaughterhouses and it can not be considered as an experimental study.

2. Material and Methods

2.1 Data collection

The initial database consisted of 137,859 records of cattle slaughtered and processed in two slaughterhouses located in Southern Chile during 2006. Incomplete records due to missing information for source of the animal, animal type or fat cover were excluded. Condemned carcasses due to cysticercoids, tuberculosis or other pathologies were also excluded from analysis. This resulted in 127,838 records that were used for prevalence estimation and further analyses. The total number of carcasses analyzed in the study represents 14% of the

total number of bovines (n=913,261; steers, cows, oxen and heifers) slaughtered in Chile in 2006 (Oficina de Estudios y Politicas Agrarias, ODEPA).

The two meat plants processed 58% of the total number of slaughtered animals in the Region Xth in Chile (n=239,746, ODEPA 2007). Slaughterhouses are identified with codes SLH1 and SLH2 to preserve anonymity. In each slaughterhouse, classification of animal type and carcass grading of the hot carcass was carried out by meat graders, who also assigned bruise grade and fat cover based on Chilean Regulations (INN Chile, 1994; 2002). In our study the term “carcass" refers to the whole body of a slaughtered animal as presented after bleeding, de-hiding and evisceration. Prevalence of bruises was defined as the percentage of carcasses presenting at least one bruise; if several bruises were present on a carcass, the meat grader only registered the most severe one.

The following variables and categories within variables were recorded for each carcass:

Bruises

A lesion qualifies as a bruise if tissues are crushed with rupture of vascular supply and accumulation of blood and serum, without cutaneous discontinuity according to INN Chile (2002). Grades of bruising of a carcass are based on the type of tissue affected:

Grade 0; no visible bruises

Grade 1; bruises in subcutaneous tissue

Grade 2; bruises in subcutaneous and muscle tissues

Grade 3; bruises in subcutaneous tissues, muscle and bone fracture.

Source of the animal

Farmer: animals transported directly from the farm to the slaughterhouse

Dealer: animals bought at the farm by a trader, association, or corporation engaged in buying, receiving, selling and transporting the animals directly from the farm or from the livestock market to the slaughterhouse

Market: animals transported from farm to a livestock market and from there transported to the slaughterhouse.

Animal type according to INN Chile (1994)

Steer: castrated male before sexual maturity with milk teeth or 2 or 4 or 6 permanent incisors teeth

Ox: mature castrated male with 8 permanent incisors teeth

Heifer: young female with at maximum 2 permanent incisor teeth

Cow: mature female with 4 or 6 or 8 permanent incisor teeth.

Hot Carcass Weight (HCW)

Carcass weight (kg) after slaughter, before chilling.

Fat cover

Measure of the thickness of external fat on the carcass by visual appraisal according to INN Chile (2002):

Grade 0: absence of fat

Grade 1: scarce

Grade 2: abundant

Grade 3: excessively abundant.

Carcass conformation rating

Good: carcass with pronounced convex profiles and excellent muscle development

Average: carcass with moderately convex profiles and good muscle development

Under average: carcass with concave profiles and very meager muscle development.

Batch number

Number assigned to a group of animals transported in the same truck unloading at the slaughterhouse and kept in the same holding pen. A batch can comprise 16 to 40 animals, of similar or different animal types. Mean stocking density commonly used during transport is 457 ± 6.6 kg/m^2 (Gallo et al., 2005), maximum stocking density allowed in Chile is 500 kg/m^2.

Season

The region of the study belongs to the category "Oceanic climate (Cfa). Precipitations are evenly distributed throughout the year, always above 40mm/month, without dry season (Kottek et al., 2006). Seasonal division is as follows:

Summer: January, February and March

Autumn: April, May and June

Winter: July, August and September

Spring: October, November and December.

Slaughterhouse

Meat plant where cattle are kept in lairage, stunned, killed and processed into meat.

Lairage time

Time between arrival of the animals and slaughter. At the time of the study, in Chile a minimum of 6 hours was compulsory unless otherwise stated by the official veterinary inspector according to MINAGRI (2004). During lairage, cattle receive water but not food unless they remain over 24 hours, in which case they receive water and hay.

Lairage time is only available for a subset of the data (n= 33,223) of SLH2. During lairage animals are kept in pens with standard conditions among slaughterhouses: 2 m^2 per animal; roofed pens with concrete floor.

pH

Measurement of pH was performed in the *longissimus dorsi* muscle at the level of the 10th rib, approximately 24 h after slaughter in refrigerated carcasses. The measurement was carried out using a portable Hanna HI99163 pH meter, and following manufacturer instructions (HANNA Instruments®, USA).

Carcasses were classified as "normal" "(pH < 5.8) or as DFD meat (pH ≥ 5.8). Data on pH classification were available for 28,627 carcasses from SLH2 for the period January - June". Carcass data of the pH group was not a part of the data of the lairage group.

2.3 Statistical analysis

Multivariable logistic regression analyses were performed on absence/presence of bruised carcasses using SAS software, version 9.1 (SAS Institute Inc., Cary, NC, USA). Bruises grade 2 and 3 were merged with bruises grade 1 in one category. The general model was:

$$Logit(\pi) = \ln\left(\frac{\pi}{1-\pi}\right) = \beta_0 + \sum \beta_i * X_i$$

Where π = the probability of presence of a bruise, β_i are the regression coefficients and X_i the explanatory variables described above (including SLH). Additionally, two models were run on subsets of the data using those records including information on lairage time or pH. Each analysis started with a univariable analysis of each predictor variable to explore the data. Next, a full model containing all predictor variables was used to estimate their effects and significance. Non-significant variables were removed one by one from the model, starting with the variable showing the highest overall p-value. The model was re-run and presence of confounding was assessed by comparing the estimates of the new model with estimates of the previous model. Confounding was deemed present when estimates changed at least 25%; confounders were forced in the model irrespective of their significance in order to obtain less biased estimates. Finally, relevant interaction terms were added to the model. Goodness-of-fit of the models was checked by the Hosmer and Lemeshow statistic. Effects of the predictor variables on the presence of bruises were expressed in terms of the odds ratios (OR) and their 95% confidence intervals (CI). An OR larger (smaller) than 1 indicates that

the bruise is more (less) likely to be present in a specific category of the predictor variable compared to the reference category.

3. Results

The prevalence of bruised carcasses identified at routine *postmortem* inspection at SLH1 and SLH2 are presented in Table 1. The prevalence was higher at SLH1 than at SLH2 (20.8% vs. 8.6 %). Carcasses with bruises grade 1 were found more frequently than carcasses with bruises grade 2 and grade 3 in both SLH1 (19.3 %) and in SLH2 (7.5%). Severe bruises (grade 3) were rarely recorded in both slaughterhouses (0.01% and 0.03% respectively). At each meat plant more female animals (58.5% and 55.3% for SLH1 and SLH2 respectively) were slaughtered than males.

Table 1. Prevalence of bruised carcasses and bruise severity in two Chilean slaughterhouses

Bruises	Grade	Prevalence (%)	
		SLH 1 (n= 37,911)	SLH 2 (n= 89,927)
No	0	79.24	91.42
Yes	1	19.82	7.50
	2	0.93	1.05
	3	0.01	0.03
	Total 1, 2 and 3	20.76	8.58

Four variables were found not to be associated with presence of bruises at the 0.05 significance level: carcass conformation, hot carcass weight, batch number and season. Moreover, two variables (sex and age) were not evaluated in the multivariable analysis due to co-linearity with the variable type of animal. Estimates of significant variables (animal type, fat cover, the interaction between SLH and source) are presented in Table 2 as odds ratios (OR) with their 95% confidence intervals (CI). From the four animal types, cows (OR= 2.6; 95% CI: 2.5- 2.7) and oxen (OR= 2.2; 95% CI: 1.9-2.5) showed an increased risk ($P < 0.0001$) for presence of bruises compared to steers. Heifers also showed an increased risk for bruising as compared to steers but this difference was less pronounced (OR= 1.2; 95% CI: 1.1-1.3). Fat cover was significantly related ($P < 0.01$) to the presence of bruises. Carcasses with scarce fat cover (grade 1) were most often (90%) reported and had the highest prevalence of bruised carcasses (11.2%). Carcasses with abundant fat (grade 2) were less frequent (4%) and showed a significantly ($p < 0.0001$) lower risk for bruising (OR= 0.8; 95% CI 0.8-0.9). However also carcasses with total absence of fat cover (grade 0), with a

frequency of 5.5% of the total population, presented a significantly ($p < 0.0001$) lower risk (OR= 0.4; 95% CI 0.4-0.5) for bruises compared to grade 1 (Table 2). Carcasses with excessively abundant fat (grade 3) were rare (0.1%), with a risk for bruising not significantly different from grades 0 and 2 (p= 0.56).

A statistically significant interaction ($p < 0.0001$) was present between the source of the animal and the slaughterhouse. The OR for SLH1 compared to SLH2 varied from 2.4 to 3.3 depending on the source of the animal (Table 2). About half of the slaughtered animals came to the slaughterhouse through a livestock market (SLH1, 53%, and SLH2, 47.1%). In both slaughterhouses animals coming through the market showed the highest bruise prevalence (OR= 1.3 compared to dealer), but the ORs for farm compared to dealer differed between SLH1 and SLH2 (OR= 1.2 and 0.9 respectively). The Hosmer-Lemeshow statistic indicated sufficient fit (p= 0.22).

A subset of the data (n= 33,223) from SLH2 was available to determine the relationship between lairage duration and the prevalence of bruised carcasses. The final model included the variables source of the animal, fat cover, animal type, lairage time and the interaction lairage time*animal type. The risk for bruises was significantly lower ($P < 0.001$) for cows and steers that spend over 12 hours in lairage compared to animals kept 6-12 hours. However, oxen showed a higher risk to present bruises when they were kept between 12 and 18 hours in lairage (Table 3). In heifers a non-significant effect of lairage time was found. The Hosmer-Lemeshow statistic yielded a p-value of 0.62.

Table 2. Risk factors for carcass bruising in Chilean cattle (n= 127,838) assessed by multivariable logistic regression

Variable	Class		n	Bruises prevalence %	OR	95% CI	P-value
Animal type	Steers		53,697	10.3	1.0	Ref.*	Ref.
	Heifers		29,184	10.2	1.2	1.1-1.3	<.0001
	Cows		42,761	18.8	2.6	2.5-2.7	<.0001
	Oxen		2,196	17.5	2.2	1.9-2.5	<.0001
Fat cover	Absence of fat (0)		7,044	0.5	0.4	0.4-0.5	<.0001
	Scarce (1)		115,482	11.2	1.0	Ref.	Ref.
	Abundant (2)		5,169	0.6	0.8	0.8-0.9	<.0001
	Excessively abundant (3)		143	0.03	0.9	0.6-1.3	ns
SLH	Farm	SLH1	9,846	17.2	3.3	3.1-3.6	<.0001
		SLH2	41,915	5.9	1.0	Ref.	Ref.
	Market	SLH1	20,102	23.3	2.4	2.3-2.5	<.0001
		SLH2	42,382	11.9	1.0	Ref.	Ref.
	Dealer	SLH1	7,963	17.3	2.5	2.2-2.8	<.0001
		SLH2	5,630	7.4	1.0	Ref.	Ref.
Source of animals	SLH1	Farm	9,846	17.2	1.2	1.1-1.3	<.0001
		Market	20,102	23.3	1.3	1.2-1.4	<.0001
		Dealer	7,963	17.3	1.0	Ref.	Ref.
	SLH2	Farm	41,915	5.9	0.9	0.8-1.0	0.03
		Market	42,382	11.9	1.3	1.2-1.5	<.0001
		Dealer	5,630	7.4	1.0	Ref.	Ref.

**Ref. stands for reference category*

Table 3. Association between presence of bruises and lairage time in Chilean cattle (n= 33,223) adjusted for source of the animals, fat cover and animal type assessed by multivariable logistic regression

Variable	Class		n	Bruises prevalence %	OR	95% CI	P-value
Lairage time (hours)	Cows	6-12	3,444	13.5	1.0	Ref.	Ref.
		12-18	3,207	10.7	0.9	0.7-1.0	ns
		18-24	1,969	8.1	0.7	0.6-0.9	0.0018
		>24 hrs	3,089	9.9	0.8	0.7-0.9	0.0079
	Heifers	6-12	2,301	7.4	1.0	Ref.	Ref.
		12-18	1,548	5.5	0.8	0.6-1.1	ns
		18-24	721	6.7	1.0	0.7-1.4	ns
		>24 hrs	1,244	6.2	0.9	0.7-1.2	ns
	Steers	6-12	2,700	6.0	1.0	Ref.	Ref.
		12-18	6,243	3.2	0.6	0.4-0.7	<.0001
		18-24	3,496	3.1	0.5	0.4-0.7	<.0001
		>24 hrs	3,604	3.3	0.6	0.5-0.7	<.0001
	Oxen	6-12	146	8.9	1.0	Ref.	Ref.
		12-18	245	15.5	2.0	1.0-3.9	0.0455
		18-24	92	9.8	1.3	0.5-3.1	ns
		>24 hrs	174	6.3	0.8	0.3-1.8	ns

**Ref. stands for reference category*

A subset of the data from SLH2, comprising 28,627 carcasses was used to determine the relationship between postmortem pH value and presence of bruises. Oxen were excluded as there were very few of them (n= 10) in this subset. From the total number of carcasses analyzed, 19.6% presented pH values higher or equal than 5.8. Twenty six percent of the bruised carcasses (n= 2,337) and 19.1% of the non-bruised carcasses (n= 26,290) presented pH values higher than 5.8. The final model included the variables source of the animal, fat cover, animal type, pH and the interaction pH*animal type. Type of animal interacted with the effect of pH, although the direction of the effect was the same within all animal types. OR's for high pH compared to low pH were 1.4, 1.2 and 1.7 within cows, heifers and steers respectively (Table 4). The Hosmer-Lemeshow statistic yielded a p-value of 0.98.

Table 4. Association between presence of bruises and carcass pH in Chilean cattle (n= 28,627) adjusted for fat cover, animal type and source of the animal assessed by multivariable logistic regression

Variable		Class	n	Bruises prevalence	OR	95% CI	p-value
pH	Cows	<5.8	3,425	12.2	1.0	Ref	Ref.
		>5.8	476	16.0	1.4	1.0-1.8	0.0235
	Heifers	<5.8	8,380	8.0	1.0	Ref.	Ref.
		>5.8	1,215	10.0	1.2	1.0-1.5	0.0382
	Steers	<5.8	11,206	5.7	1.0	Ref.	Ref.
		>5.8	3,925	10.5	1.7	1.5-1.9	<.0001

**Ref. stands for reference category*

4. Discussion

In the present study the prevalence of bruising in a data set of two Chilean abattoirs over one year period of time was quantified and related to animal characteristics and main pre-slaughter conditions, the method of selling and lairage time. Also the association between prevalence of bruising and carcass pH as an indicator for DFD meat (Silva et al., 1999) was analyzed. The results of this study can be transferred to other countries with similar pre-slaughter conditions (e.g. transport characteristics, transport distances, transport stocking densities, lairage time at the slaughterhouse). However, the protocol used for the carcass bruise evaluation is specific of Chile.

In Chile, post mortem bruising inspection in meat plants is compulsory and grading classification is based on severity of bruising and type of tissues affected (INN Chile, 2002). Bovine carcasses that present bruises grade 2 and 3 are downgraded to lower categories, but bruises grade 1 do not lead to a financial penalty. In our study, a large difference between slaughterhouses was found in the prevalence of bruised carcasses, 20.8% for SLH1 and 8.6% for SLH2 respectively. Three aspects are discussed to explain this large difference:

1) Differences in the use of the bruise grading system. Since the prevalence of carcasses with bruises grade 2 and grade 3 was low in both meat plants (0.93% and 0.01% in SLH1 and 1.05% and 0.03% in SLH2) the large difference between slaughterhouses is especially attributed to the recording of bruises grade 1 (SLH1, 19.8% vs. SLH2, 7.5%). This difference might indicate that meat graders in SLH2 focused more on bruises affecting deepest tissues and less on bruises grade 1 which were either missed or just not reported. The prevalence obtained at SLH2 is similar to previous studies carried out in Chile based on the analysis of slaughterhouse's records, which reported a bruise prevalence of 7.7% (Gallo et

al., 1999) and 13.0% (Strappini et al., 2008). However, attempts could be made to improve recording of bruises grade 1 as it gives valuable information on animal welfare conditions during the ante mortem period especially if the total number of bruises per carcass, their extent and anatomical location are also registered (Strappini et al., 2009).

2) Differences in the conditions for bruise recording. Variation in prevalence of bruises in both slaughterhouses might also be due to different conditions under which bruises are registered. The speed of the slaughter-line, the intensity of the light at the scoring location, the daily number of slaughtered animals, the number of meat graders in relation to the work load, the experience and personal interests of the grader might also affect the score of bruises. These sources of variation were also put forward as possible explanation for the variation between slaughterhouses in the submission of tuberculosis lesions in cattle (Collins, 1997; Frankena et al., 2007).

3) Differences in the source of the animals and ante mortem handling at the slaughterhouse. In SLH1, the proportion of animals coming from markets (53%) was higher compared to the proportion of animals from farms (26%) or from dealers (21%), whereas in SLH2 these proportions were 47%, 47% and 6% respectively. The higher proportion of marketed animals in SLH1 might partly explain the higher bruise prevalence in this slaughterhouse compared to SLH2. Moreover, at SLH2 more animals were from farms and had a decreased risk for bruising at this SLH as compared to an increased risk at SLH1. It is also clear that other factors at the slaughterhouse related to the handling of the animals, such as mixing of unfamiliar animals during lairage, penning of the animals at high stocking densities or use of driving elements (i.e. electric goads, sticks) (Jarvis et al., 1995; Nanni Costa et al., 2006) might differ between meat plants affecting bruise prevalence. Moreover, part of the variation between the slaughterhouses in the prevalence of bruises can be not just due to handling procedures but also due to inadequate conditions and design of meat plant facilities (McCausland and Millar, 1982).

Studies with different species and in other geographic areas reported a significant variation in bruising prevalence according to time of the year (in deer, Jago et al., 1996; in pigs, Dalla Costa, et al., 2006). In our study, no evidence was found for an effect of season of the year on bruise presence.

Regarding animal type, bruises are most frequent in cows and oxen. This finding is also supported by previous data obtained by Yeh et al. (1978), Anderson (1973) and Beasley

and Hasker (1986) who reported that mature and old animals have a higher risk for bruising in comparison to young animals. Animals with a lower economical value like cows and oxen are more likely to pass through a livestock market which implies longer transport times, multiple (un) loading procedures and more handling increasing the chances for bruises.

Fat cover showed unexpected results as the group of animals with total absence of fat cover (grade 0) showed the lowest risk for bruising. These results do not concur with those reported for sheep (Knowles et al., 1994) and deer (Jago et al., 1996). However, Chandra and Das (2001) working with buffaloes, also members of the subfamily *Bovinae*, found that weak (skinny animals with a prominent skeletal structure) and heavy animals (mostly fatty animals) presented less bruises than average animals. These results might be due to the fact that weak and heavy animals put up less resistance to being moved, especially during unloading, than animals of normal body condition.

Animals sold through market have the highest risk of showing bruises (OR= 1.3) compared to animals delivered by dealers for both SLH's. Weeks et al. (2002) found similar results and stated that the most likely explanation is differences in handling. According to Gregory (1996) bruising is evidence of poor animal handling. The more an animal is handled, the greater the chance on injuries leading to carcass bruising. However, the effect of source of the animal is modified by the effect of the slaughterhouse due to the fact that in SLH2, animals coming directly from farms have slightly lower risk (OR= 0.9) compared to animals delivered by dealers. Reasons for this difference between slaughterhouses are not clear.

Lairage time is significantly associated with the presence of bruises. Cows and steers have a reduced risk (OR < 1.0) to develop bruises when they spent more hours in the holding pens. Holding conditions at the slaughterhouse may contribute to the development of bruises during early lairage time. In Chile, a common practice after the arrival of the animals at the meat plant is to regroup and mix unfamiliar individuals. When cattle are regrouped, the new-comer animals have to find their position in the social order again, and with that the number of agonistic encounters is increased (Brakel and Leis, 1975; Cockram and Corley b, 1991) as well as the probability of bruises. Recently, it was reported (Liste et al., 2009) that the extent of bruising in rabbit carcasses varied between lairage groups, being significantly higher in the short lairage group (2 hours) than in the long lairage group (8 hours). The researchers concluded that the higher level of bruises may be associated to the pre-slaughter handling of

more reactive animals. A better understanding of why cattle kept in lairage for longer periods of time show the lowest risk for bruises is still needed. If we are able to assess age of the bruises this may help to pinpoint the time when the bruise has been inflicted (at the slaughterhouse or before).

The analysis of pH data showed a positive relationship between postmortem pH category and presence of bruises (OR varies from 1.2 to 1.7, depending on animal type). When animals are stressed, glycogen reserves can be depleted and hence a higher pH can be expected (McVeigh and Tarrant, 1982). It is likely that improper handling, long transport, prolonged fasting times and physical injuries result in increased stress and in depletion of muscle glycogen before slaughter. After slaughter, production of lactic acid is limited by the low levels of glycogen values preventing that the pH of the meat drops. McNally and Warriss (1996) reported that 48% of heavy bruised carcasses presented pH values over 5.8 compared to 11.5% in light bruised carcasses. Both studies therefore suggest a strong relationship between stress conditions, bruises and pH carcass values.

The present study based on data from two Chilean slaughterhouses over a full year period reveals that:

- Cows and oxen are more than two times prone to show bruises than young animals, heifers and steers. This may ask for special care during pre-slaughter procedures.
- The route through the livestock market is evidently associated with an increased risk of bruising.
- Animals that have longer lairage times (over 12 hours) have a significantly reduced risk for bruises, except for oxen.
- Presence of bruises is significantly associated with increased carcass pH values of the carcasses.

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Chapter 4

Intra- and inter-observer reliability of a protocol for post mortem evaluation of bruises in Chilean beef carcasses

A.C. Strappini[a,b], K. Frankena[c], J.H.M., Metz[d] and B. Kemp[a]

[a] *Adaptation Physiology Group, Wageningen University, P.O. Box 338, 6700 AH, Wageningen, The Netherlands*
[b] *Institute of Animal Science, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Casilla 567, Isla Teja, Valdivia, Chile*
[c] *Quantitative Veterinary Epidemiology Group, Wageningen University, P.O. Box 338, 6700 AH, Wageningen, The Netherlands*
[d] *Department of Agrotechnology & Food Sciences, Farm Technology Group, Wageningen University, P.O. Box 17, 6700 AA, Wageningen, The Netherlands*

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Abstract

The objective of this study was to test the reliability of the post-mortem evaluation of bruise characteristics using a newly extended protocol. Intra-observer reliability of the observations was assessed with three meat graders, each of whom blindly examined 50 photos of bovine carcasses in two sessions, with one week in between. The agreement was 'substantial' for number of bruises per carcass, and size and shape of the bruises; whereas it was 'moderate' for colour and severity grade. Inter-observer reliability of the observations was assessed with four meat graders who each examined the same 46 carcasses at the slaughter-line. Inter-observer agreement for number of bruises per carcass ranged between observer pairs from 'fair' to 'moderate' The agreement for number of bruises scored per anatomical site was 'very low' for all observers. The most concordant bruise feature was size, followed by colour and shape. The bruise scoring protocol was associated with high level of intra-observer reliability and low inter-observer reliability. A further calibration of the observer judgments is needed to reach a more uniform scoring of the observers.

Keywords: *bruises; carcass evaluation; observer reliability*

1. Introduction

A reliable bruise scoring protocol for carcasses evaluation at slaughter houses is important for two main reasons. Firstly, for meat quality assessments and valuing carcasses, particularly when high-value sections of the animal body are bruised which lead to the downgrading of the carcass (Warriss, 2010). Secondly, for animal welfare assessment when the harmful situations that animals underwent prior to slaughter must be identified (Algers, 2006). In both cases, information on the reliability of bruise scoring protocol is generally lacking (Suanes et al., 2003).

The aim of this study was to determine the reliability of a post mortem evaluation of bruises based on a variety of visual characteristics by estimating the intra- and inter-observer agreement.

2. Material and Methods

The number of bruises per carcass and the number of bruises per anatomical site were assessed. Carcass bruises were evaluated according to a new protocol detailed in Strappini et al. (2012). Briefly, the protocol defines a bruise as a lesion where tissues were crushed with a rupture of vascular supply and an accumulation of blood and serum without discontinuity of the skin (Anderson and Horder, 1979; INN Chile, 2002). The following data were collected for each bruise: the anatomical site (butt, rump-loin, ribs, forequarter, back, pin and hip), the size (small, medium, large) according to the Australian Carcass Bruising Scoring System (ACBSS; Anderson and Horder, 1979), the severity grade (grade 1, grade 2 and grade 3), based on the Chilean bruising carcass-grading standard (INN Chile, 2002) as well as the shape (irregular, linear, circular, mottled and tram-line) and the colour (red, bluish or dark coloured and yellow-orange).

Board-certificated meat graders (INN Chile, 2002) -with 1 to 5 years field experience- were selected on availability to execute the observations. Before, they were trained for using the protocol by means of visual aids (photographs and videos) and direct evaluation of carcasses at the slaughter-line.

2.1. Intra-observer reliability

Intra-observer reliability was assessed with three trained meat graders. Each observer individually scored 50 colour photos (15 x 20cm) in two sessions, one week apart to prevent

recall bias. The carcass photos were judged within one minute which resembled the speed of the slaughter-line.

Bruise scores in session 1 and 2 were used to determine kappa for categorical variables while the within-observer reliability on number of bruises per carcass was assessed using the intra-class correlation coefficient ICC (3, 1) (Shrout and Fleiss, 1979).

$$ICC(3,k) = \frac{BMS - EMS}{BMS + (k-1)EMS}$$

Where k is the number of sessions (2), *BMS* is the between-session mean square, and *EMS* is the within-session (residual) mean square. Weighted kappa values (K_w) were calculated for ordinal variables (Cohen, 1968). The analysis considered two main questions:

1. Do the observers identify the same bruises in session 1 and in session 2
2. Do the observers assign the identified bruises with the same scores for grade, size, shape, and colour in both sessions.

2.2. Inter-observer reliability

Four trained meat graders participated in this part of the study. At the slaughterhouse, the observers scored blindly 46 carcasses for presence/absence of bruise. The time spent on each individual carcass was approximately 1 minute.

Based on the evaluation of the carcasses at the slaughter line the inter-observer reliability was assessed. The analysis considered two main questions:

1. Do the four observers record the same number of bruises per carcass and per site,
2. Do they identify the same bruises and assign the same score to the bruises regarding grade, size, shape, and colour.

Again, ICC (3,*k*) was used to assess agreement between all observers (k= 4) and between each pair of observers (k= 2) for the number of bruises. Weighted kappa was used to determine the agreement between observer pairs for bruise characteristics.

3. Results

3.1. Intra-observer reliability

In session 1, observer 1 classified 8 carcasses as non-bruised, observer 2, 6 carcasses and observer 3, 7 carcasses. In session 2 the observers deemed the same 8, 6 and 7 carcasses as non-bruised. The number of bruises per carcass ranged from 0 to 18 per carcass. The average number of bruises per carcass scored in session 1 (2) was 2.6 (2.7), 3.8 (4.7) and 5.6 (6.9) for observer 1, 2 and 3 respectively. The ICC for the number of bruises per carcass scored in two intermittent sessions ranged between 'moderate agreement' for observer 2 (ICC= 0.78) to 'almost perfect agreement' for observers 1 (ICC= 0.95) and 3 (ICC= 0.91).

Kappa values for bruise characteristics grade, size, shape and colour are presented in Table 1. The reliability estimates were in general very high for the three observers. The mean intra-observer agreement between sessions for grade was 'moderate' (k= 0.59), but with a large variation between observers (range: 0.32-0.78). Observer 1 and 2 showed 'substantial agreement' and observer 3 'fair agreement'. Size and shape of the bruises showed 'substantial agreement' for the three observers and ranged between 0.71-0.78 for size, and 0.68-0.79 for shape respectively. For colour, the agreement between sessions was 'substantial' for the two experienced meat graders (0.67 and 0.69) and 'fair' for observer 3 (0.35).

Table 1. Kappa estimates for grade, size, shape, and colour of carcass bruises as scored by three observers in two intermittent sessions

Variable	Categories	Observer 1			Observer 2			Observer 3		
		Session 1	Session 2	Kappa (95%CI) *Kw** (95%CI)	Session 1	Session 2	Kappa (95%CI) *Kw* (95%CI)	Session 1	Session 2	Kappa (95%CI) *Kw* (95%CI)
Grade				0.78 (0.64-0.93)			0.68 (0.58-0.77)			0.32 (-0.02-0.67)
	1	59 (68.6)	59 (68.6)		195 (73.3)	172 (64.7)		376 (98.2)	378 (98.7)	
	2	27 (31.4)	27 (31.4)		71 (26.7)	94 (35.3)		7 (1.8)	5 (1.3)	
Size				0.64 (0.51-0.78)			0.67 (0.57-0.76)			0.70 (0.62-0.78)
				0.71 (0.60-0.83)			0.74 (0.67-0.82)			0.78 (0.72-0.84)
	Small	23 (26.7)	16 (18.6)		42 (25.3)	33 (19.9)		128 (51.2)	122 (48.8)	
	Medium	33 (38.7)	41 (47.7)		57 (34.3)	59 (35.5)		66 (26.0)	66 (26.4)	
	Large	30 (34.9)	29 (33.7)		67 (40.4)	74 (44.6)		57 (22.8)	62 (24.8)	
Shape				0.73 (0.48-0.98)			0.58 (0.45-0.71)			0.70 (0.62-0.79)
				0.79 (0.57-1.00)			0.68 (0.56-0.81)			0.79 (0.71-0.86)
	Circular	2 (2.3)	1 (1.1)		48 (28.9)	33 (19.9)		68 (27.2)	48 (19.2)	
	Irregular	77 (89.5)	79 (91.9)		107 (64.5)	121 (72.9)		147 (58.8)	170 (68.0)	
	Lineal	2 (2.3)	2 (2.3)		3 (1.8)	4 (2.4)		15 (6.0)	12 (4.8)	
	Mottled	0 (0.0)	0 (0.0)		2 (1.2)	1 (0.6)		1 (0.4)	1 (0.4)	
	Tram-line	5 (5.8)	4 (4.7)		6 (3.6)	7 (4.2)		19 (7.6)	19 (7.6)	
Colour				0.67 (0.52-0.83)			0.69 (0.57-0.80)			0.35 (0.24-0.46)
	Red	37(43.0)	41 (47.7)		66 (39.8)	52 (31.3)		200 (80.0)	152 (60.8)	
	Bluish	49 (57.0)	45 (52.3)		100 (60.2)	114 (68.7)		47 (18.8)	89 (35.6)	
	Yellow	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)		3 (1.2)	9 (3.6)	

**weighted kappa*

3.2. Inter-observer reliability

Out of the 46 carcasses evaluated, 45 carcasses were scored as bruised. One carcass was scored as non-bruised by all four observers and therefore excluded from the analysis. The number of bruises per carcass as scored by the four observers ranged from 2-15 for observer 1, 1-12 for observer 2, 1-8 for observer 3 and 1-9 for observer 4. The agreement between all 4 observers for the number of bruises per carcass was 'moderate' (ICC= 0.70). The level of agreement regarding the number of bruises per carcass per observer pair is presented in Table 2, and shows that observer-pair 1-4 had the lowest performance (fair agreement, ICC= 0.43), and observer-pair 3-4 -composed by the two more experienced meat graders- the best performance ('moderate agreement', ICC= 0.80).

Table 2. Level of agreement (ICC) for number of bruises scored by observer-pairs

Observer-pair	ICC	Average per observer	Strength of agreement
1-2	0.70	6.9-5.5	Moderate
1-3	0.48	6.9-4.5	Fair
1-4	0.43	6.9-4.2	Fair
2-3	0.69	5.5-4.5	Moderate
2-4	0.77	5.5-4.2	Moderate
3-4	0.80	4.5-4.2	Moderate

In total, 487 times an anatomical site with one or more bruises was identified: 169 reported by observer 1, 125 by observer 2, 95 by observer 3 and 98 by observer 4. 'Slight agreement' (ICC= 0.35) was found for number of bruises scored per site for the group of observers as a whole. The sites butt, rump-loin, pin and hip scored less than 35 observations per observer pair and were excluded therefore from further calculations. There was large variation in the number of bruises scored per site between observer-pairs. Pair 1-3 showed 'virtually no agreement' (ICC= 0.05) whilst the observer-pairs 2-4 and 3-4 showed 'fair agreement' (ICC= 0.45 and ICC= 0.50, respectively).

Kappa values for the bruise characteristics grade, size, shape and colour are presented in Table 3. All observer-pairs had reliability estimates smaller than 0.61 (substantial agreement). Colour of the bruises was the characteristic with the lowest Kappa for all observer pairs and ranged between 0.16 and 0.39 (from' slight' to 'fair' agreement). Size of the bruises showed the highest level of agreement and ranged between 0.43 and 0.56 ('moderate' agreement).

Table 3. Inter-observer reliability for scores of bruises

Bruise characteristic	Obs. pair	Number of observations	*K*	Lower limit	Upper limit	*Kw*	Average *K*	Strength of agreement
Grade	1-2	253	0.16	0.01	0.32	-	0.27	Fair
Size	1-2	253	0.46	0.38	0.55	0.54		
Shape	1-2	253	0.22	0.12	0.33	0.38		
Colour	1-2	253	0.22	0.12	0.32	-		
Grade	1-3	179	0.46	0.29	0.62	-	0.41	Moderate
Size	1-3	179	0.47	0.36	0.58	0.52		
Shape	1-3	179	0.43	0.29	0.56	0.51		
Colour	1-3	176	0.28	0.18	0.38	-		
Grade	1-4	112	0.58	0.41	0.74	-	0.37	Fair
Size	1-4	112	0.56	0.44	0.69	0.62		
Shape	1-4	112	0.17	0.08	0.26	0.24		
Colour	1-4	107	0.16	0.02	0.30			
Grade	2-3	179	0.19	0.02	0.35	-	0.32	Fair
Size	2-3	179	0.50	0.40	0.61	0.56		
Shape	2-3	179	0.24	0.11	0.36	0.39		
Colour	2-3	176	0.36	0.23	0.50	-		
Grade	2-4	112	0.23	0.07	0.39	-	0.39	Fair
Size	2-4	112	0.55	0.42	0.68	0.62		
Shape	2-4	-	-	-	-	-		
Colour	2-4	107	0.39	0.22	0.56			
Grade	3-4	112	0.46	0.27	0.64	-	0.30	Fair
Size	3-4	112	0.43	0.30	0.57	0.51		
Shape	3-4	112	0.13	0.06	0.19	0.22		
Colour	3-4	107	0.16	-0.03	0.35	-		

4. Discussion

The intra-observer reliability in the scoring for presence and number of bruises per carcass was evaluated for three observers and showed to be very high. Scoring of the grade and colour of the bruises, showed a ‘substantial intra-observer agreement’ for two observers and ‘fair agreement’ for the third, less experienced observer. Grade and colour could be difficult features to categorize from small-sized photos as done in this study, especially regarding the depth of the damage or the type of affected tissues. The values of Kappa and weighted Kappa statistics for the bruise characteristics size and shape showed a ‘high agreement’ between sessions for all observers. So, although some variation between the scores of grade and between the scores of

colour was observed, the results indicate that observers had overall a high level of consistency within their own evaluation of carcasses.

The inter-observer reliability for the total number of bruises ranged from 'fair' to 'moderate agreement'. For carcass grading purposes a 'moderate agreement' in the score of the number of bruises may be considered as acceptable. But for animal welfare evaluations this outcome is not 'optimal'. The number of bruises present on a carcass reflects the level of harm suffered by the animal in the *ante-mortem* period (Grandin, 2000). Therefore, when multiple observers are included in a study to judge carcasses, good inter-observer reliability is needed to assess the actual impact on animal welfare.

We found 'slight inter-observer agreement' (ICC= 0.35) in the number of bruises scored per anatomical site and 'fair inter-observer' agreement (k= 0.48) for the severity grade of the bruises. Our findings are in agreement with data obtained at Uruguayan slaughterhouses by Huertas et al. (2003) who found 'fair' inter-observer agreement for site and for severity grade (k= 0.5 and k= 0.48 respectively) using similar scoring criteria. To determine whether a bruise is shallow or extends to deeper tissues can be difficult at the slaughter-line, especially when it is not feasible to cut the carcass to appreciate the precise depth of a bruise.

The most concordant bruise feature was size, followed by colour and shape. Source of variation in the scores of the observers may be associated to the experience of the observer, speed of the slaughter line and the difficulty to score while the carcass is moving. More training on these features could result in a more uniform scoring between observers.

Conclusion

The assessment of carcass evaluation following a training period and using a detailed protocol which included the description of the characteristics of the bruises was associated with a high level of intra-observer reliability and a low inter-observer reliability. Further observer calibration is needed to improve agreement.

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Chapter 5

Characteristics of bruises in carcasses of cows sourced from farms or from livestock markets

A.C. Strappini[a,b], K. Frankena [c], J.H.M Metz [d], C. Gallo [b] and B. Kemp [a]

[a]*Adaptation Physiology Group,* [c]*Quantitative Veterinary Epidemiology Group, Department of Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH, Wageningen, The Netherlands*
[d]*Farm Technology Group, Department of Agrotechnology & Food Sciences, Wageningen University, P.O. Box 17, 6700 AA, Wageningen, The Netherlands*
[b]*Instituto de Ciencia Animal, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Casilla 567, Isla Teja, Valdivia, Chile*

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Abstract

Bruises in cattle develop after the application of force, and they provide evidence for sub-optimal animal welfare. The aim of the study is to describe the gross characteristics of bruises in cows arriving at the slaughterhouse directly from farms or through the livestock market. The number of bruises and their distribution on the carcass as well as their severity, shape, size and colour were assessed post mortem in a slaughterhouse in Chile. Two hundred fifty eight cow carcasses were evaluated, and a total of 846 bruises were found on 243 of the carcasses. Cows that had passed through a livestock market (M-carcasses) had in total, 563 bruises (mean 3.8 bruises/carcass, s.d. 2.0) whereas cows transported directly from farms (F-carcasses) had in total 283 bruises (mean 2.5 bruises/carcass, s.d. 1.8). The backs of F-carcasses had twice as many bruises as M-carcasses (32.9% and 16.2%, respectively), whereas bruises in the rib area were more frequently observed in M-carcasses (13.1%) than in F-carcasses (8.1%). Superficial bruises (grade 1) were the most frequently observed (66.2% of all bruises). Regarding the size of the bruises, 64 (7.6%) was classified as large, 271 (32.0%) as medium and 511 (60.4%) as small. Irregularly shaped bruises were most frequent (91.1%, n= 771), followed by linear (3.8%, n= 32), circular (3.1%, n= 26) and tramline shaped bruises (1.9%, n= 16). The latter were noticed only in M-carcasses, which may indicate that these animals were beaten more frequently with sticks or other rod-shaped objects. Fresh, bright red-coloured bruises were found more frequently on all the animals (69.5% from farms and 70.5% from market) compared with bluish (29.7% and 29.3% respectively) and yellow bruises (0.4% and 0.2%). The method of selling was significantly associated with the number of bruises on the carcass ($p<0.001$) and the anatomical site ($p<0.05$), but not with the severity, shape and colour of the bruises. Increased fat coverage reduced the severity of bruises ($p<0.001$).

This study shows that, in Chile, market animals have more bruises than those sourced directly from farms, and their distribution is different. More information about the causes of infliction may help reduce bruises and it may also improve their welfare. Further studies are needed to elucidate whether the causes of the high bruising in the case of animals passing through markets are related only to extra handling (repeated loading, unloading, transportation, eventual mixing) or to the way of handling by personnel and inadequate design.

Keywords: *bruises, markets, farms, cattle, welfare*

Implications

Bruises are subcutaneous lesions that become visible after the skin has been removed during the dressing of a carcass, and they are indicators of sub-optimal welfare conditions during the pre-slaughter period. Characteristics of bruises such as size, colour, shape and grade may provide information about the extent of sub-optimal welfare. In our study, we compared bruises on the carcasses of cows obtained from two types of sources: either sourced from a livestock market or coming from farms. The results show that most carcasses have bruises and that marketed cows have more bruises than cows sourced directly from farms, but the bruise characteristics were not significantly different between market and farm cows. The results suggest that the pre-slaughter handling of the cows was not optimal and further research should be undertaken into the causes of bruising.

1. Introduction

Bruises are subcutaneous lesions that may vary in number, distribution, severity, extent, colour and shape. Because of the type of hair coat and the thickness of the skin, bruises in cattle are only clearly visible after slaughter and de-hiding. Bruises are important in relation to carcass quality (meat quality), and therefore, carcasses are classified according to the severity of the lesions on the slaughter-line. In Chile, when a bruise affects muscle tissue and the damage is considerable, the bruised area is trimmed and the carcass is downgraded, leading to economic losses.

Bruises are also an important source of information about animal welfare, and they are described as such in pigs (de Koning, 1985; Faucitano et al, 1998; Lambooij, 2000), poultry (Mayes 1980; Gregory, 1994; Nijdam et al., 2004; Broom and Reefmann, 2005), rabbits (Liste et al., 2009), deer (Jago et al., 1996; Matthews, 2000), sheep (Cockram and Lee, 1991; Jarvis and Cockram, 1994; Tarumán and Gallo, 2008) and horses (Grandin et al., 1999). In cattle, the observation of bruising is used to determine whether animal welfare is sub-optimal (Strappini et al., 2009). For carcass evaluation, identification of only severe bruises is sufficient, but for welfare assessment, an accurate quantification of the number of bruises, their location on the carcass, and the diversity of bruise characteristics is needed to identify the impact on welfare and, potentially, to unravel the cause and the moment of infliction of the bruise.

The shape of a bruise is often directly associated with the causative event. For instance, circular shaped bruises, which are deep and small in extent, are most likely caused by horns (Grandin, 2000); parallel red bruises with a tram-line appearance are likely caused by sticks

(Weeks et al., 2002) and mottled bruises can be the result of the use of pointed sticks (Gallo, 2009).

Although the colour of a bruise is associated with the time of occurrence of the bruise, there is no general agreement among authors as to the exact age associated with each colour and the sequence of colour changes. Rough estimates are provided by Gracey and Collins (1992), who reported that a bright red bruise is likely to be up to 10 hours old, a dark red bruise is approximately 24 hours old, and a yellow bruise is more than 3 days old. Hamdy et al. (1957) reported a different sequence of colour changes in cattle bruises: red coloration from 15 minutes to 2 days, green from day 3 to day 4 and yellow and orange from days 4 to 6. McCausland and Dougherty (1978) found that a yellow coloration appeared by 48 hours.

The severity of a bruise is related to the force applied and the part of the body that was damaged. Bruises inflicted over the *gluteus* muscles of cows are deeper than those inflicted over the *lumbo-dorsal fascia* (Hamdy et al., 1957). Overall, the severity of a bruise depends greatly on the thickness and density of the affected tissue and its vascularity.

The method of animal selling is commonly associated with the number of bruises and their distribution on the carcass (Eldridge et al., 1984; McNally and Warriss, 1996; Weeks et al., 2002). Higher numbers of bruised carcasses are reported for marketed animals than for animals transported directly from the farm (Weeks et al., 2002; Strappini et al., 2009). Marketed animals present more bruises on the hip, buttocks and rump-loin than animals transported directly from farms to the slaughterhouse (Wythes et al., 1982; Blackshaw et al., 1987; Jarvis et al., 1995). These animals are exposed to extra loading and unloading procedures and to group mixing, which is likely associated with more bruises. A recent epidemiological study carried out in Chile has shown that animals from markets are at higher risk of being bruised than animals coming directly from farms (OR of 1.4) (Strappini et al., 2010b). In that study, bruises on carcasses were recorded by the Chilean grading system (INN, 2002), in which only the most severe bruise per carcass is considered and information about other bruises, further characterisation of bruises and their anatomical location is missing.

The aim of this study is to provide a detailed description of the number and characteristics of bruises on cattle carcasses and to relate these parameters to the source of the animals, that is, directly transported from farm to slaughterhouse, or passing through a livestock market first.

2. Material and methods

Data collection

The study was carried out at a slaughterhouse in Valdivia, Chile, between February and April 2009. The carcasses of cows were used in our study due to the relatively high bruise prevalence (18.8%) within this category (Strappini et al, 2010b). The cows were of the dairy type, and they were defined as mature female bovines with 4, 6 or 8 permanent incisor teeth. Maximum transport distance travelled by the animals - from market or farm to the slaughterhouse - was 200 km. General handling conditions for cattle at this slaughterhouse considered a lairage time between 12 and 18 hours; groups of cattle were driven by personnel trained in animal handling from the lairage pens through a curved race to the stunning box. The animals were stunned using a non-penetrating captive bolt pistol, immediately chained by their left hind leg, shackled onto the slaughter-line and ex-sanguinated. The slaughterhouse processes on average 350 cattle per day, and the average slaughter-line speed is 50 animals per hour.

A total of 265 cow carcasses were intermittently inspected on the slaughter-line over 11 days. The carcasses were selected from the daily slaughter program of the slaughterhouse in consideration with information reported by the Chilean Ministry of Agriculture (ODEPA, 2009) that about 40% of the cows come directly from farms and about 60% are traded through a livestock market before arrival at the meat plant. Seven carcasses had incomplete data, leaving 258 carcasses for analysis, of which, 111 were from animals transported directly from the farm (F-carcasses) to the slaughterhouse and 147 were from animals that had passed through a livestock market (M-carcasses).

For each individual cow carcass included in our study, the following information was available:

Age: ‘young’ having 4 or 6 permanent incisor teeth and ‘old’ having 8 permanent incisor teeth (INN, 2002).

Hot carcass weight (HCW): carcass weight (kg) after slaughter and removal of the hide, head, and legs at the *metacarpus* (foreleg) and *metatarsus* (hind leg) phalange joints and of all internal organs, but before chilling.

Fat coverage: degree of thickness of external fat on the carcass, assessed by visual appraisal according to INN Chile (2002); grade 0: absence of fat; grades 1, 2, and 3: scarce, abundant, and excessively abundant fat coverage, respectively.

Protocol for bruise evaluation

The evaluation of cow carcasses for the presence of bruises and bruise characteristics was carried out by a meat grader and veterinarian with five years of experience. This observer was

placed after the de-hiding point and before the carcass-splitting point along the slaughter-line, allowing the entire carcass -hanging by both hind legs- to be easily observed.

The protocol for the post-mortem evaluation was based on the Australian Carcass Bruising Scoring System, ACBSS (Anderson and Horder, 1979) and the Chilean bruising carcass-grading standard (INN Chile, 2002). The parameters of shape and colour of bruises were added. We defined a bruise as a lesion where tissues were crushed with a rupture of vascular supply and an accumulation of blood and serum without discontinuity of the skin (Anderson and Horder, 1979; INN Chile, 2002).

For each carcass, the presence of bruises (yes or no) was recorded first. If bruises were present, the number of bruises per carcass and the number of bruises per anatomical site were assessed. Next, each bruise present on the carcass was evaluated by registering its anatomical site, size, severity, shape and colour. The original ACBSS score sheet for half a carcass was extended to allow the complete registration of bruises for the entire carcass. The anatomical site of the bruise was recorded according to the ACBSS, and the carcass was divided in seven sites as codes 1 to 7 (Figure 1):

1. Butt: hindquarter distal, *Mm. gluteobiceps, semitendinosus, gluteus medius*
2. Rump-loin: *Mm.obliquus externus abdominis* , *transversus abdominis*
3. Rib: *Mm. intercostalis externi* and *interni, tranversus thoracis*
4. Forequarter: *Mm. trapezius, latissimus dorsi, infraspinatus, supraspinatus, deltoideus*
5. Back: the spinal column and adjacent muscles from the neck to the butt of the tail
6. Pin: *tuber isquiadicum*, insertion of *Mm*. *semitendinosus* and *gluteobiceps*
7. Hip: *tuber coxae* of *ilium*, insertion of muscles *Mm. gluteus profundus* and *tensor fasciae latae.*

If a bruise covered more than one site, it was indicated as “multiple site”.

The size of the bruise was assessed on the basis of its diameter according to the ACBSS: small: ≥2 and ≤8 cm; medium: >8 and ≤ 16cm; large: > 16 cm. When a bruise was not circular, the diameter was measured as the longest length of the lesion. To assist with the visual appraisal of the bruises, a disc indicating circular areas of 2, 8 and 16 cm was used.

The severity of the bruise was scored by the observer according to the Chilean bruising grading classification (INN Chile, 2002): grade 1: only subcutaneous tissue affected; grade 2: as grade 1, but with muscle tissue affected; grade 3: as grades 1 and 2, but with the presence of broken bones.

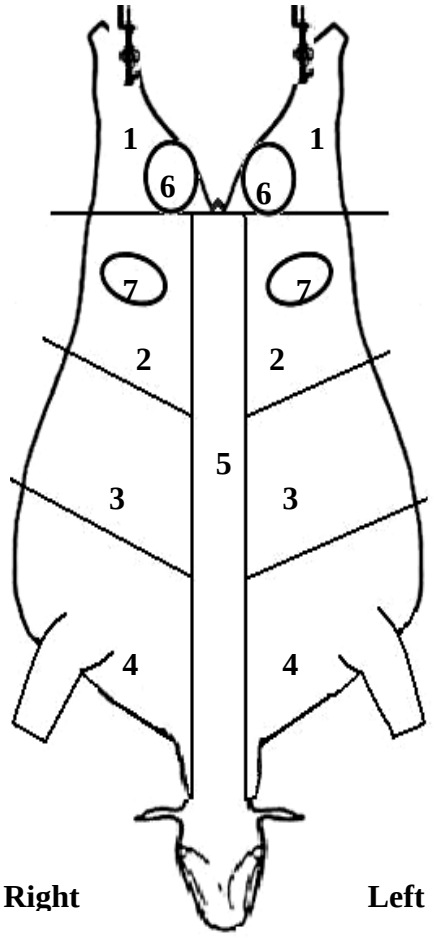

Figure 1. Bruise scoring sheet, numbers indicate the location (sites) of the bruises on the carcass being 1. butt; 2. rump-loin; 3. ribs; 4. forequarter; 5. back; 6. pin and 7. hip

The shape of the bruise, defined as the characteristic pattern or form of a bruise, was classified according to previous studies (Grandin, 2000; Weeks et al., 2002; Strappini, 2010 a). The following shapes were distinguished: circular: a bruise shaped like or nearly like a circle; linear: a non-circular bruise with one dimension (length) longer than the other (width); tramline: two parallel linear bruises separated by a paler undamaged area; mottled: the bruised area appears spotted or blotched; and irregular: a bruise without clear dimensions and with uneven margins (Figure 2).

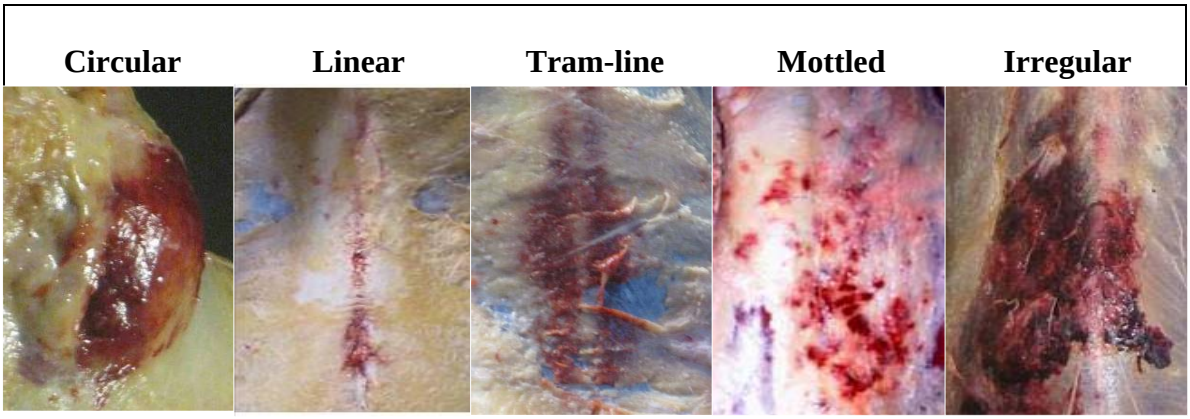

Figure 2. Shape of bruises as defined in this study

The colour of the bruise was scored as follows: 'red', corresponding to a fresh bruise; 'bluish or dark' for an old bruise; and 'yellowish/orange' for a very old bruise.

Bruises smaller than 2 cm, blemished injection sites, and reddening lesions that looked like bruises, located on the left hind leg - more likely caused after stunning by the tightening of the shackle chain - were not recorded.

Statistical analysis

Differences between carcasses arriving via markets or directly from farms were tested using GLM (Gaussian distribution) for the number of bruises per carcass and by generalised estimated equations (GEE), which specified a binomial distribution with a logit link function for the number of bruises per site and the bruise characteristics (grade, size and colour). As we were not interested in carcass-specific estimates, but in the total population of all carcasses, we preferred a population-average model (GEE) over a subject-specific model (MIXED). In all GEE models, the carcass was included as a random effect because multiple bruises per carcass might not be independent; the exchangeable correlation structure was specified. The main independent variable was origin of the carcass (from farm or market). The covariates were the age of the cow, the hot carcass weight and the degree of fat coverage. Initially, all of these variables were included in the model. The covariates were removed one by one, the order being based on the highest p-value, until all variables were significant or were deemed a confounder for the main effect. Confounding was defined to be present when the removal of a covariate resulted in a change of the estimate for the origin of the animal of more than 25%. Finally, 2-way interaction terms for the remaining variables were evaluated. Combinations of bruise characteristics (colour, shape, severity grade and size) and their relation to the origin of the carcass were evaluated similarly. Fitting of the GLM models was performed by graphical inspection of the residuals.

The fit of the binary models was determined by assessing the Hosmer-Lemeshow statistic using ordinary logistic regression. All analyses were performed using SAS (SAS Institute Inc., Cary, NC, USA).

Results

Origin of carcasses

A total of 258 carcasses were assessed with regard to bruises, 147 arrived from markets and 111 from farms. From 140 carcasses it was known from which market (n= 4, range 18-55) they came and for 109 carcasses from which farm (n= 16, range 1-24). Number of bruises per carcass did not differ significantly between the 4 markets (p= 0.15) and also not significantly between the 16 farms (p= 0.17). Therefore we considered both populations of carcasses, coming either from market (M-carcasses) or from farm (F-carcasses), to be homogeneous. Information about transport conditions (i.e. distance, stocking density, duration of the journey, and truck characteristics) was incomplete and excluded from the analysis.

Carcass and bruise characteristics

Table 1 presents the characteristics of the M- and F-carcasses. The age (young, old) differed significantly between the carcass origins, showing that a greater percentage of the cows classified as 'old' were sourced from a market than directly from a farm (Chi square, $p<0.01$). Animals with abundant or excessively abundant fat coverage (grades 2 or 3) were not observed. For grades ‘zero’ and 1, the fat coverage was not significantly different between the M- and F-carcasses (Chi square, p= 0.23). For HCW, the F-carcasses were, on average, 28.3 kg heavier than the M-carcasses (GLM, linear regression, $p<0.001$).

Table 1. Baseline characteristics of dairy-type cows arriving from market (n=147) or directly from the farm (n=111).

		Origin	
Characteristic	Level	Market (%)	Farm (%)
Age[1]	Young	5 (3.4)	15 (12.9)
	Old	142 (96.6)	96 (87.1)
Fat coverage[2]	0	36 (24.5)	20 (21.5)
	1	111 (75.5)	91 (78.5)
	2	0 (0.0)	0 (0.0)
	3	0 (0.0)	0 (0.0)
Mean hot carcass weight (kg ± SD)		232 ± 33	260 ± 55

[1] young: animal with 4 or 6 permanent incisor teeth; old: animal with 8 permanent incisor teeth
[2] fat coverage 0: denotes absence; 1: scarce; 2: abundant and 3: excessive

Number and location of bruises

There were 846 bruises on the 258 inspected carcasses, 238 (92.2%) of the carcasses were bruised and 20 carcasses (7.8%) were recorded as non-bruised. The carcasses sourced directly from farms were more often free of bruises (n= 13 out of 111, 11.7%) than the carcasses sourced from a livestock market (n= 7 out of 147, 4.8%) (Fisher's exact p-value: 0.058). The frequency distribution of the number of bruises per carcass is shown in Figure 3. Carcasses from animals hat had passed through a livestock market showed, in total, 563 bruises (mean 3.8 bruises per carcass (s.d. 2.0); median 4.0; range 0-9), while carcasses from animals transported directly from farms had, in total, 283 bruises (mean 2.5 bruises per carcass (s.d. 1.8); median 2.0; range 0-8). The number of bruises per carcass was significantly related to the origin of the carcass, with M-carcasses showing an average of 1.4 more bruises than F-carcasses (GLM, $p<0.001$, residuals normally distributed).

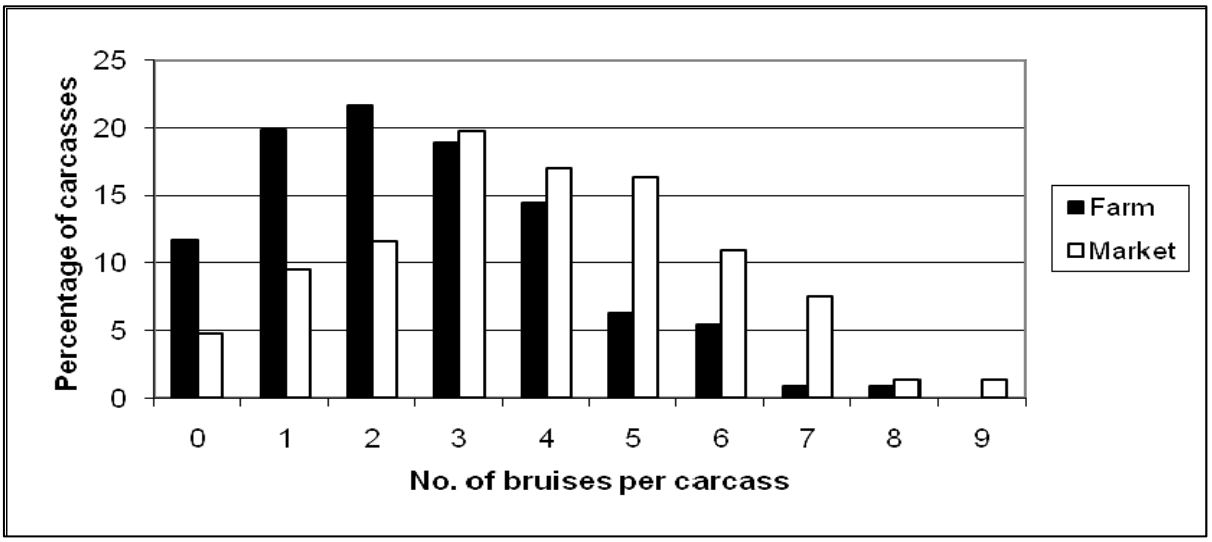

Figure 3. Number of bruises per carcass of cows from markets (n= 147) or arriving directly from the farm (n=111).

The distribution of the bruises over the carcass sites (Figure 4) shows that the pin of the carcass was the most frequently bruised area (26.5% of all bruises) followed by the back (21.8%), while the butt was the least affected site (0.4%). The back of F-carcasses had a 2 times greater probability of being bruised than M-carcasses (32.9% and 16.2%, respectively), while bruises in the rib area were more frequently observed in M-carcasses (13.1% and 8.1%, respectively) (Figure 4). The 846 bruises on the 238 bruised carcasses were distributed over 630 sites. The majority of these sites showed one bruise (n= 440); 167 showed 2 bruises; and 23 sites

showed 3 or more bruises. For the analysis (Table 2), the number of bruises per site was recoded as 1 bruise per site or 2 or more bruises per site. The final GEE model included only the site and source of the carcass; the odds ratio (OR) of having 2 or more bruises per site was 1.5 (95% CI: 1.0-2.1, p-value: 0.06) for M-carcasses compared with F-carcasses. The ORs of having 2 or more bruises in a site were significantly higher for 'back', 'pin' and 'multiple sites', compared with the 'rump-loin' area. The exchangeable working correlation was 0.04, and the HL-statistic in the ordinary logistic regression showed sufficient fit (p-value: 0.85).

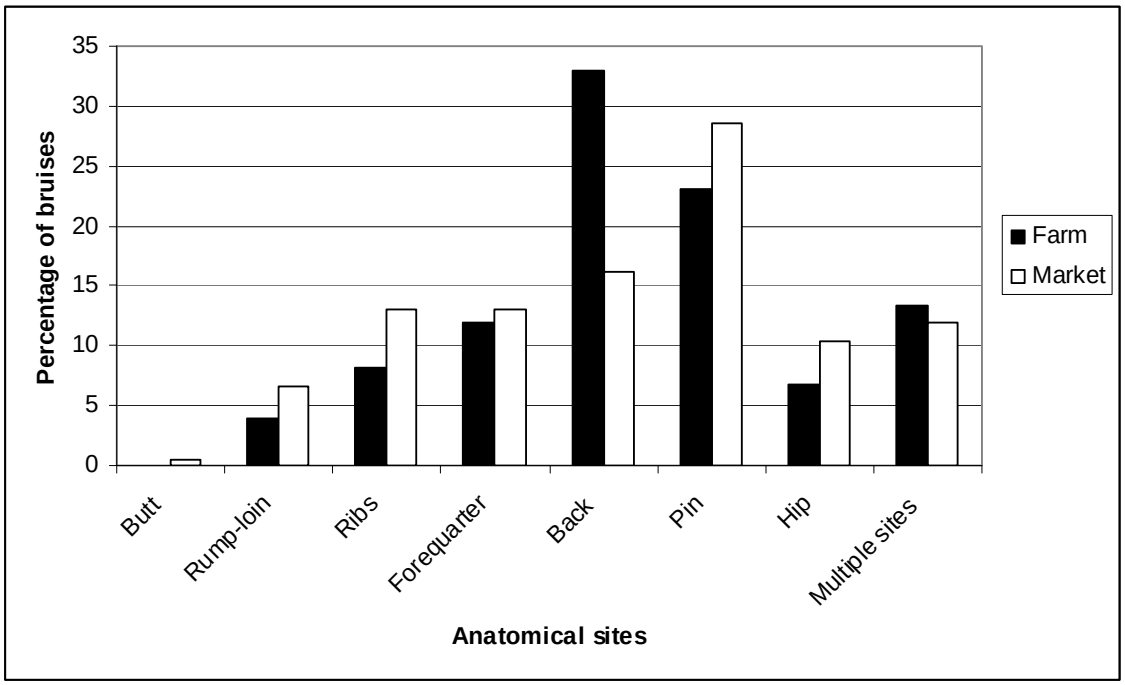

Figure 4. Number of bruises per site of cow carcasses from markets (n= 147) or arriving directly from the farm (n=111).

Bruise characteristics

All models using binary data showed sufficient fit as determined by the Hosmer-Lemeshow statistic ($p>0.05$).

Grade

Superficial bruises (grade 1) were most frequently observed (66.2% of all bruises) (Table 3). Bruises of grade 2 –muscular damage- were observed in 142 carcasses, totalling 286 bruises (33.8% of all bruises). There were no cows presenting severe bruises (grade 3) with the presence of broken bones. In the GEE model, fat coverage was the only significant variable related to grade (OR for fat coverage 1 compared to 0 was 0.7, 95% CI 0.5-0.9, p-value 0.01); (Table 2).

The OR for M-carcasses versus F-carcasses was 1.2 (95% CI 0.9-1.6, p-value 0.13, exchangeable working correlation 0.17).

Table 2. Final models estimates (GEE) for number of bruises per site and bruise characteristics

Dependent variable	Class	N	%[1]	OR[2]	P-value
No. of bruises per site[3] (2 or more vs 1)	Rump-loin	41	17.1	1.0	Ref.
	Ribs	76	21.1	1.4	0.54
	Forequarter	86	19.8	1.3	0.56
	Back	134	32.1	2.7	0.03
	Pin	157	44.0	4.2	<0.01
	Hip	62	22.6	1.6	0.39
	Multiple sites	72	33.3	2.7	0.05
	Farm	217	26.3	1.0	Ref.
	Market	411	32.4	1.5	0.06
Grade (2 vs 1)	Fat coverage 0	130	44.6	1.0	Ref.
	Fat coverage 1	716	31.8	0.7	0.01
	Farm	283	29.7	1.0	Ref.
	Market	563	35.9	1.2	0.13
Size (large vs small+medium)	Farm	283	9.5	1.0	Ref.
	Market	563	6.6	0.7	0.12
Size (large+medium vs small)	Farm	283	35.7	1.0	Ref.
	Market	563	41.6	1.3	0.19
Colour[4] (red vs blue)	Farm	282	70.2	1.0	Ref.
	Market	562	70.6	1.0	0.89

[1] % indicates the % of sites/bruises showing the first level of the dependent variable
[2] OR estimates were adjusted for presence of more than one bruise per carcass
[3] butt excluded from the analysis as only 2 bruises were observed on all butts
[4] 2 yellow bruises excluded from the analysis

Size

Of the 846 bruises, 64 (7.6%) were classified as large, 271 (32.0%) as medium and 511 (60.4%) as small (Table 3). One or more large bruises were observed on 54 carcasses: 23 (20.7%) were on F-carcasses and 31 (21.1%) were on M-carcasses. In the GEE analysis of this ordinal variable, the proportional odds assumption did not hold, and 2 separate analyses were performed comparing large versus small or medium bruises and large or medium versus small bruises, respectively. In the first GEE model, the odds for showing large bruises versus not large (small or medium) were lower for M-carcasses compared with F-carcasses, though they were not statistically significant (OR= 0.7, 95% CI 0.4-1.1, p-value 0.12, exchangeable working correlation <0.01). In the second model, the odds of showing large or medium sized bruises

were increased for M-carcasses compared with F-carcasses, but they were also not significant (OR= 1.3, 95% CI 0.9-1.8, p-value 0.19, exchangeable working correlation was 0.09).

Table 3. Frequencies of the bruise characteristics according to the source of the cows (n=846).

			Farm	Market
Variable	Category	Total n (%)	n (%)	n (%)
Grade[1]	1	560 (66.2)	199 (70.3)	361 (64.1)
	2	286 (33.8)	84 (29.7)	202 (35.9)
Size	Small	511 (60.4)	182 (64.3)	329 (58.4)
	Medium	271 (32.0)	74 (26.2)	197 (35.0)
	Large	64 (7.6)	27 (9.5)	37 (6.6)
Shape	Circular	26 (3.1)	9 (3.2)	17 (3.0)
	Linear	32 (3.8)	12 (4.2)	20 (3.6)
	Tram-line	16 (1.9)	0 (0.0)	16 (2.8)
	Mottled	1 (0.1)	0 (0.0)	1 (0.2)
	Irregular	771 (91.1)	262 (92.6)	509 (90.4)
Colour	Bright red	595 (70.3)	198 (70.0)	397 (70.5)
	Blue	249 (29.4)	84 (29.7)	165 (29.3)
	Yellow	2 (0.2)	1 (0.4)	1 (0.2)

[1]Grade score 1: subcutaneous tissue damaged, grade 2: subcutaneous and muscular tissue damaged

Shape

Irregularly shaped bruises were by far the most frequent (91.1%, n= 771), followed by linear (3.8%, n= 32), circular (3.1%, n= 26) and tramline bruises (1.9%, n= 16) (Table 3). Mottled bruises were observed only once and were therefore excluded from further analyses. Tramline shaped bruises were noticed only in M-carcasses. The shapes of the bruises were significantly associated with the origin of the carcasses (Fisher's Exact test, p= 0.019). The significance was entirely due to the absence of tramline shaped bruises in F-carcasses. No further multivariable statistics were performed for this nominal variable.

Colour

The majority of bruises (n= 595, 70.3%) had a bright red colour; 29.4% (n= 249) were bluish; and 2 bruises (0.2%) were yellow (Table 3). A multivariable analysis using the GEE models revealed no significant variables. The OR of M-carcasses versus F-carcasses using the colour 'red' (yes/no) as a dependent variable was 1.0 (95% CI 0.7-1.4, p-value 0.89; Table 2).

Combinations of characteristics

The most frequent combination was small, red and irregular bruises grade 1 (n= 300, 35.5%) followed by medium, red and irregular bruises grade 1 (n= 130, 15.4%, Table 4). Only combinations of characteristics that were observed at least 10 times or more were analysed further. The final GEE model showed that the OR for presenting medium, blue and irregular bruises of grade 2 was significantly higher for M-carcasses (OR= 2.05; p-value: 0.03, Table 4) than for F-carcasses.

Table 4. Frequency of combinations of bruise characteristics according to the source of the cows (n= 846)

Combination Colour-shape-grade-size	Total N (%)	Farm N (%)	Market N (%)	OR[1]	P-value
Red-irregular-1-small	300 (35.5)	108 (38.2)	192 (34.1)	0.90	0.30
Red-irregular-1-medium	130 (15.4)	40 (14.1)	90 (16.0)	1.13	0.46
Red-irregular-1-large	29 (3.4)	11 (3.9)	18 (3.2)	0.82	0.62
Red-irregular-2-small	42 (5.0)	11 (3.9)	31 (5.5)	1.42	0.37
Red-irregular-2-medium	33 (3.9)	11 (3.9)	22 (3.9)	1.00	0.99
Blue-irregular-1-small	40 (4.7)	18 (6.4)	22 (2.6)	0.61	0.17
Blue-irregular-2-small	87 (10.3)	31 (11.0)	56 (10.0)	0.90	0.72
Blue-irregular-2-medium	71 (8.4)	14 (5.0)	57 (10.1)	2.05	0.03

[1] F-carcasses were taken as reference

Discussion

In Chile a large number of cows (228,386 cows in 2009) are sold through livestock markets (ODEPA, 2009). This type of sale involves extra transport, (un)loading, handling and mixing with unfamiliar animals, thus increasing the risk for bruising. The present study investigated the effect of the source of cows for slaughter (via market or directly from farm) on the number and characteristics of bruises on their carcasses.

Our data show that carcasses from animals sourced from markets and those sourced directly from farms both have a high probability of having bruises (95.2% and 88.3%, respectively). These findings are in line with those reported by Weeks et al. (2002), who found that 71.0% of the animals that had passed through a market had a bruised carcass compared to 53.7% of the animals from farms. The average number of bruises per carcass was also significantly ($p<0.001$) higher in M-carcasses (3.8) than in F-carcasses (2.6). The high number of bruised carcasses and the number of bruises per carcass indicates sub-optimal welfare conditions (Strappini et al., 2009). The higher number of bruises found in M-carcasses than in F-carcasses could be associated with the quality of the human-animal relationship in the livestock

markets, the multiple journeys, extra (un)loadings and an extra period of animal handling (Knowles 1999). However, further research is needed to unravel the specific causes.

There were significant differences between F- and M-carcasses in the distribution of bruises over the carcass. Animals from farms more often presented bruises on the back, whereas animals from markets more often presented bruises on the ribs, hip and the pin site (Figure 4). In the UK, Jarvis et al. (1995) and Weeks et al. (2002) reported more bruises on the hip, back, shoulders and butt in market animals. The differences in the distribution of bruises on the carcasses in Chile compared with those in the UK are probably due to differences in the design of facilities, handling procedures and the type of animals used. The distribution of the bruises can provide information about their causes. According to Grandin (2000), back bruises are most likely caused by equipment problems such as collisions with structures and they could be an indicator of rough handling. Pin and rib bruises may be attributable to hitting against structures such as races, corners and badly maintained sides of vehicles, but also to the use of driving instruments such as sticks, which are commonly observed to be used at Chilean markets (Gallo, 2005). Tramline bruises were observed only on M-carcasses in the rib area. This type of bruise resembles the object that inflicted the lesion, as in the case of wooden sticks (McNally and Warriss, 1997; Weeks et al., 2002). Our results suggest that market animals in Chile have more bruises that are inflicted by handling and the use of driving instruments than farm animals.

Bruises are a source of pain (Gregory, 2007), and therefore, the degree of damage may be indicative of how much the animal has suffered. The average distribution of bruises over grading categories was similar for animals from the market and from the farm. However, the severity of bruises was associated with the fat coverage of the animal, regardless of origin. Animals with a poor body condition score without fat coverage had a higher risk of presenting severe bruises with muscular damage. The presence of some fat coverage seems to protect the animal from the occurrence of severe bruises. Therefore, animals with a poor body condition score should be handled with extra care.

In the present study, most bruises had a bright red colour (Tables 2 and 3), which is characteristic of the so-called fresh bruises (Grandin, 2000). This colour confirms that most bruises were recent and were produced during the *ante mortem* period, probably caused during transit, at unloading, or during lairage at the slaughterhouse. However, the determination of the age of a bruise based solely on colour is a subjective assessment and can be inaccurate.

Considering that in Chile a common practice is to deliver the animals at the livestock market in the morning, that auction takes place in the afternoon and that the premises have to be cleared of animals before the end of the same day, all the above mentioned handlings could have occurred between 24 and 48 hours previous to slaughter in animals from both sources. Therefore, a more precise estimation of the age of the bruises combined with the timing of pre-slaughter events would provide better information on where and when the welfare conditions of the animal were sub-optimal.

Bruises appear on the carcass as a combination of colour, shape, extent and severity. Red, irregular, small and grade 1 bruises were recorded most often, but their occurrence was not affected by the source of the cows. Blue, irregular, medium and grade 2 bruises were found more often in M-carcasses than in F-carcasses (Table 4). This result suggests that M-carcasses present more severe, older and larger bruises than F-carcasses, which is consistent with the extra handling of the cattle passing through the markets and extra time being exposed to bruising events. However, as we analysed 8 characteristic combinations of bruises, this result should be taken with caution because multiple testing of a single significant result could be coincidental.

The prevalence of bruises contrasts with previous studies (Gallo et al., 1999; Strappini et al., 2010b) – performed in the same area of the country- using the Chilean bruising protocol (INN Chile, 2002), where only 7.7% and 12.3% of the carcasses were reported as bruised, respectively. This large difference is due to the Chilean system, in which only the most severe bruise is reported when there are multiple bruises, while in the ACBSS, all bruises observed are reported. In practice, small bruises of grade 1 are rarely reported at Chilean slaughterhouses (Strappini et al., 2010b), as they are of no consequence for the final price of the carcass. Because of this practice, the Chilean system presents constraints to the identification of problems linked to the welfare of the animal.

In conclusion, this study shows that animals sourced directly from farms or through markets both have a high level of bruising as compared to findings reported previously in Chile using the official Chilean bruise protocol. These results also raise the question of the causes of these bruises and their relationship with animal welfare. As bruises were more prevalent in M-carcasses than in F-carcasses, this suggests that at least part of the reason for the difference in the prevalence of bruising is due to a higher exposure to handling procedures, as cows from markets are likely to have been handled more than those sourced directly from farms. Further studies are needed to elucidate whether the causes of the higher prevalence of bruising in cows

passing through markets are only related to extra handling (repeated loading, unloading, transportation, mixing), or also to inadequate design of market facilities (loading/unloading pens, races, holding pens, weighing points) and to the way in which animals are handled at the market. The gross characteristics of the bruises are a valuable tool to identifying and evaluating potential sub-optimal welfare conditions during the pre-slaughter period. Therefore, it is advisable that a detailed evaluation of bruises be included in animal welfare assessment protocols.

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Chapter 6

Bruises in culled cows: when, where and how are they inflicted?

A.C. Strappini[a,b], J.H.M Metz[d], C. Gallo[b], K. Frankena[c], R. Vargas[b], I. de Freslon[b], and B. Kemp[a]

[a]*Adaptation Physiology Group,* [c] *Quantitative Veterinary Epidemiology Group, Department of Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH, Wageningen, The Netherlands*
[b]*Instituto de Ciencia Animal, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Casilla 567, Isla Teja, Valdivia, Chile*
[d]*Farm Technology Group, Department of Agrotechnology & Food Sciences, Wageningen University, P.O. Box 17, 6700 AA, Wageningen, The Netherlands*

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Abstract

In Chile, cow carcasses present the highest bruise prevalence compared to other cattle categories; however the causes of the bruises are frequently unknown. In this study, 52 cull cows were transported to the slaughterhouse in three batches under similar transport conditions. A combination of direct observation and video analyses was used to determine moment, pre-slaughter stage and cause of potential bruising events during the period from loading on the farm till stunning at the slaughterhouse. After slaughter, number of bruises, location on the carcass and characteristics of the bruises were assessed. Seventy eight bruises were observed on 37 carcasses. Fifty-two bruises were linked back to their causal event. Results showed that 46 % of these bruises were a result of interactions between animal and facility and most of them were inflicted in the stunning box, 27 % of the bruises originated from animal-animal interactions and were mostly inflicted during lairage, another 27 % was a result of human-animal interactions and were mostly inflicted during loading and unloading of animals. The percentages of potential bruising events resulting in a bruise were 43, 9 and less than 1 % for animal-facility, human-animal and animal-animal interactions, respectively. Most bruises on the back site were inflicted when the animal was in the stunning box (91%), while bruises on the pin site were mostly (75%) inflicted during loading at the farm. One may conclude that in relative short journeys (≤ 4 hours) directly from farm to the slaughterhouse and long lairage times (>12 hours) most bruises are the result of circumstances at the slaughter house. A substantial amount of these bruises could be avoided by proper animal handling and adequate stunning facilities.

Keywords: *bruises, moment of infliction, bruising event, pre-slaughter*

Implications

Bruising in cattle can occur at any point of the meat chain. Research regarding the moment of infliction and the type of bruising events will help to identify the factors and circumstances that contribute to impaired animal welfare and in consequence, this knowledge can be used to install measures to prevent bruise occurrence.

Introduction

In Chile, cows are primarily kept for milk production and when culled transported to the slaughterhouse to enter the meat chain (Pinedo *et al.,* 2011). More than 200,000 cows are yearly slaughtered (ODEPA, 2011) representing over 25% of the total slaughtered cattle in the country. Cow carcasses present the highest bruise prevalence compared to other cattle categories (Strappini *et al.,* 2010); however the causes of the bruises are generally unknown. Bruises may negatively affect meat quality and carcass value, and are at the same time indicators of impaired welfare during the pre-slaughter stages.

During transport and at the slaughterhouse animals may be exposed to incidents that potentially lead to bruises (Nanni Costa *et al.,* 2006) which become visible on the carcass after the animal is de-hided. Jarvis and Cockram (1994) introduced the term 'potentially bruising event' to describe a traumatic action that could cause a bruise to the animal. For cattle, several potentially bruising events were indicated, such as forceful handling (Grandin, 2000), inadequate design of the slaughter facilities (Weeks *et al.,* 2002), mounting and butting between animals (Warriss, 1990), especially during loading (Minka and Ayo, 2007), and rough driving during transport (Broom, 2003). However, the contribution of each of these potential bruising events to actual bruising of the carcass is not well documented.

For using bruises as indicators for impaired welfare it is relevant to know the characteristics of the bruises. But for full understanding of the impact on welfare and for prevention, the events that caused the bruises should be known as well. Video recording and/or direct observation can be used to investigate the handling and behaviour of the animals during the pre-slaughter period and therewith identify potential bruising events. Subsequently, the observations can be related to the presence and anatomical location of bruises observed *post mortem* to assess the cause of a bruise (Cockram and Lee, 1991; Nanni Costa *et al.,* 2006). In this study, potentially bruising events were identified during the pre-slaughter stages and subsequently their relationships with type and anatomical location of the bruises observed on the carcass were examined.

The aim of this study was to identify 'when' bruises were inflicted (time before slaughter), 'where' (in which pre-slaughter stage) and 'how' they were inflicted (type of potential bruising event) in Chilean culled cows.

Material and methods

Animals

The study was conducted during April and May 2010 (autumn season), in Southern Chile (Región de los Ríos). Fifty two culled dairy cows were transported in batches from three local farms (one batch from each farm) under standard commercial conditions to the slaughterhouse. The cows were Black and Red Friesian crosses, the majority was hornless (n= 48; 92.3%) and 'old' (96.2%, defined as animals with 8 permanent incisor teeth). At post mortem evaluation, 70.0% of the carcasses were deemed as presenting 'scarce fat cover' (grade 1, INN Chile, 2002) and the rest as 'with absence of fat cover' (grade 0, INN Chile, 2002). The mean hot carcass weight was 261.5 ± 40.8 kg.

Transport conditions, loading and unloading

The truck used to transport the cows from the farm to the local slaughterhouse was a medium-size vehicle with open roof, as commonly used for commercial livestock transportation (Chile MINAGRI, 2005). The animal compartment was 8.50 m long, 2.80 m wide and 1.80 m high. The rear door consisted of two adjacent but independent panels opening outwards. The first transport concerned 15 cows and a distance of 115 km, the second 19 cows and 100 km, and the third 18 cows and 130 km. The animals were not restrained whereas the space allowance per animal was between 1.25 and 1.58 m^2 / animal.

Before loading at the farm, the animals of the same batch were individually identified with a legible sign using fluorescent paint.

During loading the rear doors of the truck were opened outwards making contact with the walls of the fixed ramp available at the farm, demarcating in this way the loading zone. The stockpersons of the farm together with the truck driver drove the animals onto the truck using wooden sticks with sharpened points and an electric prod.

When the vehicle arrived at the slaughterhouse, the personnel of the abattoir unloaded the animals with the help of the truck driver. No driving aids were used. Next, the animals were driven onto a scale and weighed as one batch.

Lairage conditions

After weighing, the cows of each farm were kept in lairage in roofed pens (one pen per batch) where they received water *ad libitum* but no feed. The pens had a solid-concrete floor without bedding. The space allowance in the pen was approximately 2 m^2 / animal.

After lairage, the animals were moved from their holding pen to a single-line squeeze chute. No driving aids were used. The chute was curved, had solid walls and its narrow width prevented animals from turning. At the end of the chute a manually operated vertical rising-gate ('guillotine-type'), allowed the entrance of the first animal of the line into the stunning box. Two persons moved the cows into the stunning box, using flags or an electric prod. All the cows were stunned using a non-penetrating captive bolt pistol and then ex-sanguinated.

Recording and analysis of potential bruising events throughout stages

During transport and lairage, continuous video recording of the animals was carried out using a multi-camera system (waterproof metal shell, model IP 67 infrared, DVR Toshiba mobile anti-shock). Two cameras were located in the animal compartment of the truck, one on the front and the other on the back part. The cameras recorded and displayed all the activities of the animals in real-time. At the slaughterhouse, two other infrared cameras were set up in the holding pen where cattle remained in lairage. Potential bruising events in other stages - during loading and unloading, and during the entrance of the animals in the chute and inside the stunning box- were registered by direct observation by one observer (background in veterinary science, skilled in behavioural observations).

The digitalized videos were analyzed by another observer (background in animal science, skilled in behavioural observations). For the assessment of the potential bruising events each animal was followed separately using focal animal sampling technique, FAS (Martin and Bateson, 1996).

From the video recordings and direct observations the moment of infliction (hour and minutes), stage of infliction (e.g. lairage or loading), anatomical area (frontal, dorsal -proximal or distal - lateral, abdominal, ventral or caudal), and side affected (right or left) were assessed for each observed potential bruising event (Figure 1).

Orientation of anatomical areas used for video and direct observations

Frontal (F)	Anterior area of the body
Caudal (C)	Posterior area of the body situated towards the tail
Dorsal (D)	Back area of the body, opposed to ventral. Dorsal- proximal (Dp) described the site towards the head, dorsal-distal (Dd) the site towards the tail of the animal
Ventral (V)	Area pertaining to the abdomen, opposed to dorsal
Abdominal (A)	Area between the chest and the pelvis
Lateral (L)	Area toward the left or right side of the body

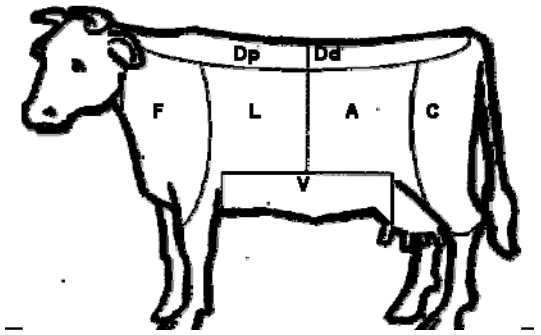

Figure 1. Orientation of anatomical areas used for video and direct observations

Types of potential bruising events recorded

Three categories of potential events were distinguished (Protocol A, Table S1 in online supplementary material).

1. Human-animal interaction: actions performed by a person to an animal that involved the use of force or electric shock (i.e. hitting, poking, pricking and use of electric prod).

2. Animal-animal interaction: forceful actions between two animals in close proximity involving physical contact. The animal that performed the action was called ‘actor’ and the animal receiving the aggression was the ‘recipient’. The focus was on the recipient animal that was mounted, butted or stamped.

3. Animal-facility interaction: impacts on the animal due to direct contact with permanent or temporary structures of the physical environment (i.e. facility). For instance, impacts with a gate, collisions against a door or impacts with the floor of the truck when the animal falls down during transport. The combination of the events 'falling down' and subsequently 'being stamped' in the truck was considered as two independent events when the impacts affected different anatomical sites.

Table S1
Protocol 'A' used to record potential bruising events by direct observation and video tracking during loading, transport, unloading, lairage, at the entrance of the stunning box and inside the stunning box.

Type of interaction	Potential bruising events	Description
Human-animal	Hit with blunt object	A person hits the animal with a hard object
	Hit with stick	A person beats the animal with a stick or similar
	Poked with electric prod	A person applies electricity to shock the animal
	Pricked with sharp object	A person sticks a pointed object in the animal's body
Animal-animal	Butted with head	An animal is bumped with force by a hornless co-specific using the head
	Butted with horns	An animal is bumped with force by a horned co-specific using the head and the horns
	Stamped on	An animal steps forcibly onto other animal' back
	Mounted	An animal stands (recipient) while other animal (actor) clasps its forelegs on both sides of the standing animal and positions its fore body on the back and rump of the mounted animal
Animal-facility	Impact with blunt object	A blunt object falls onto the animal body
	Collision	An animal impacts against a hard object i.e. fence or door
	Impacts with cutting object	An animal impacts against a sharp edge and causes a cutting njury
	Falls down	An animal loses balance and drops from standing position to sternal or decubitus recumbence

Post mortem evaluation of bruises on the carcass

After removing the hide and before splitting - approximately 30 minutes after bleeding - the 52 entire carcasses (hanging by both hind legs) were evaluated for presence of bruises by one trained board certified (Chile MINAGRI, 2009) veterinary meat grader. Bruises were defined as lesions where tissues were crushed with rupture of vascular supply and accumulation of blood and serum (INN Chile, 2002). The carcass was virtually divided into various areas and the observer was instructed to record the number and the characteristics of the bruises: size, severity grade, shape and colour (Protocol B, Table S2 in online supplementary material). The head and the belly of the animal were excluded.

Table S2
Protocol 'B' used for post mortem bruise evaluation at the slaughter-line (according to Strappini et al., Animal, 2012).

Variable	Category	Definition
Anatomical site	1.Butt	Hindquarter distal area, *Mm. gluteobiceps, semitendinosus, gluteus medius*
	2.Rump-loin	*Mm.obliquus externus abdominis*, *Transversus abdominis*
	3.Rib	*Mm. intercostalis externi* and *interni, tranversus thoracis*
	4.Forequarter	*Mm. trapezius, latissimus dorsi, infraspinatus, supraspinatus, deltoideus*
	5.Back	The spinal column and adjacent muscles from the neck to the butt of the tail
	6.Pin	*Tuber isquiadicum*, insertion of *Mm. semitendinosus* and *gluteobiceps*
	7.Hip	*Tuber coxae* of *ilium*, insertion of muscles *Mm. gluteus profundus* and *tensor fasciae latae.*
	Multiple site	Bruise covering two or more sites
Size	Small	≥2 and ≤8 cm;:
	Medium	>8 and ≤ 16cm;
	Large	> 16 cm
		*When a bruise is not circular, the diameter should be measured as the longest length of the lesion
Severity grade	Grade 1	Only subcutaneous tissue affected
	Grade 2	Subcutaneous and muscle tissue affected
	Grade 3	Subcutaneous and muscle tissue affected with presence of broken bones
Shape	Circular	A bruise shaped like or nearly like a circle
	Linear	A non-circular bruise with one dimension (length) longer than the other (width)
	Tram-line	Two parallel linear bruises separated by a paler undamaged area
	Mottled	The bruised area appears spotted or blotched
	Irregular	A bruise without clear dimensions and with uneven margins
Colour	Red	Fresh bruise
	Bluish	Old bruise
	Yellowish	Very old bruise

Statistical analyses

All bruises detected on the carcass were tried to trace back to a potential bruising event, as registered on the basis of the video recordings and direct observations, to determine the causal event. If no event was linked to a bruise then the causal event was recorded as "unknown".

The association between pre-slaughter stage and type of bruising event on one hand and the anatomical distribution of the bruises over the carcass and their visual appearance (grade, size, shape and colour) on the other hand were tested using contingency tables (Fisher exact test). The relative risk (expressed as Odds Ratio) of developing a bruise after a human-animal, animal-animal or animal-facility interaction was assessed using logistic regression in SAS, version 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

Pre-slaughter conditions

Duration (hours and minutes) of the five pre-slaughter stages (loading, transport, unloading, lairage and permanence in the stunning box) is presented in Table 3. The duration of the total pre-slaughter period- from the farm to the slaughter and before bleeding- was for each of the batches somewhat over 20 hours. Most of the time was spent in lairage at the holding pen (average 19 h 20 min). Loading and unloading took nearly the same time (average 11 and 8 min) while the time in the stunning box was shortest (1 min).

Table 3. Time duration of the pre-slaughter stages loading, transport, unloading, lairage and permanence in the stunning box for 52 cows transported in three batches

Batch	Loading (min)	Transport (h:min)	Unloading (min)	Lairage (h:min)	Stunning box (min)	Total time (h:min)
1	10	4:15	10	16:10	1	20:46
2	17	2:10	3	18:36	1	21:07
3	7	4:12	10	22:15	1	26:45
Average	11	3:12	8	19:20	1	22:52

Occurrence of potential bruising events

A total of 1,792 potential bruising events were recorded for 52 cows. Ninety one percent of the events were observed during lairage, 5.4% in the stunning box at the slaughterhouse, 2.5% at loading, 0.4% during transport and 0.5% at unloading.

Figure 2 shows that during loading, unloading and in the stunning box most of the events were of human-animal type (97.7%, 75.0% and 51.5%, respectively). At lairage and during transport animal-animal interactions were most frequently found (99.7% and 71.4%, respectively).

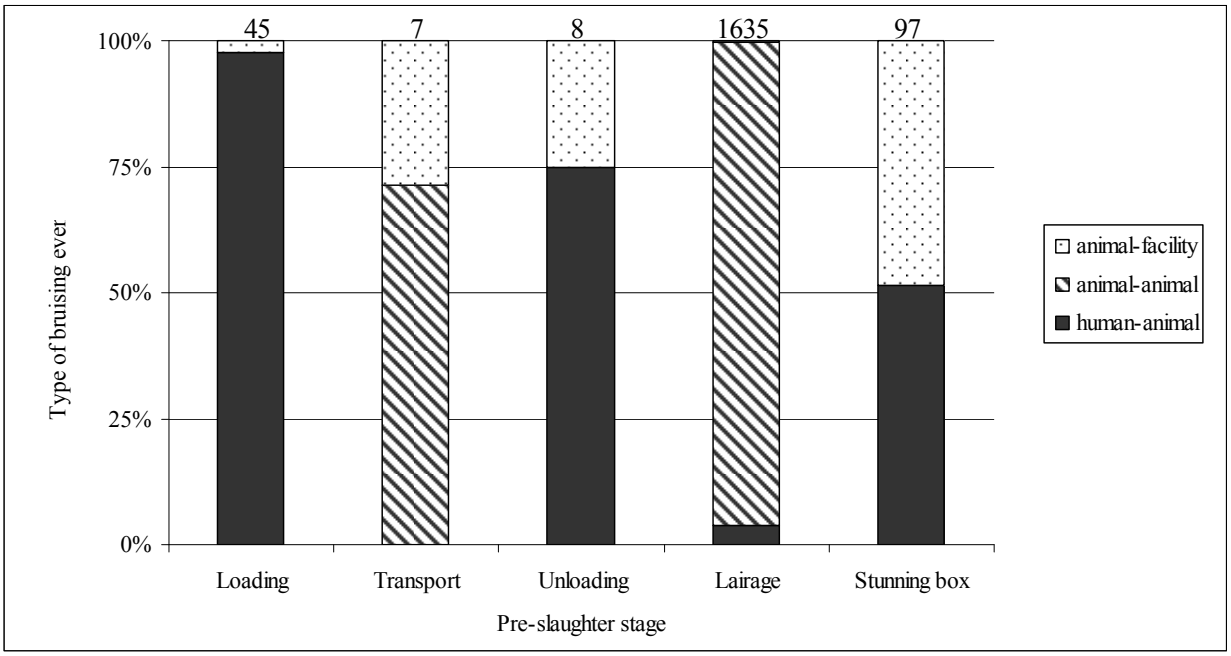

Figure 2. Human-animal, animal-animal and animal-facility interactions during loading, transport, unloading, lairage and in the stunning box (n = 1,792 events of 52 cows)

Occurrence and visual appearance of bruises

From the 52 carcasses evaluated, 37 (71.0%) presented a total of 78 bruises. The mean number of bruises per bruised carcass was 2.1±1.2 (range 1-5). Animals of batch 1 presented 13 bruises while it was 34 for batch 2, and 31 for batch 3. The bruises were most frequently found on the back (30.8%) and the pin (24.4%) sites of the carcasses (Table 4). Within back, the dorsal distal area was most often bruised (83.3%). No bruises were observed on the butt site of the carcasses.

Of all bruises 80.8% were of grade 1, most had an irregular shape (94.9%), had red colour (57.7%), and showed a diameter between 2 and 8 cm (55.1%). The bruises of the three batches did not significantly differ in grade, size, shape and colour (Fisher exact *P*-value= 0.29, 0.41, 1.00 and 0.30, respectively).

Table 4. Anatomical distribution of 78 bruises as found on 37 carcasses and 52 bruises with cause known

Anatomical site	Bruises n (%)	
Butt	0 (0.0)	0 (0.0)
Rump-loin	2 (2.6)	2 (3.8)
Ribs	6 (7.7)	3 (5.8)
Forequarter	11 (14.1)	4 (7.7)
Back	23 (29.5)	13 (25.0)
Pin	19 (24.4)	17 (32.7)
Hip	8 (10.3)	4 (7.7)
Multiple sites	9 (11.5)	9 (17.3)
Total	78	52

Moment, stage of infliction and type of potential bruising event

For 52 bruises (66.7%) out of the 78 observed bruises it was possible to identify the moment, stage of infliction and the type of event that caused the lesion. Thirty eight percent of the bruises were inflicted within 1 hour from slaughter (Fig. 3-A). The majority of the bruises (67.3 %) occurred at the slaughterhouse (during unloading, lairage and in the stunning box, Fig. 3-B). The 'impact with a blunt object' caused 36.5% of the bruises (Table 5), and rough handling such as 'pricked with a sharp object' contributed with 23.1%, whilst 9.6% of the bruises were caused by interactions between animals like 'butted with horns'(Fig. 3-C). Three animals lost balance and 'fell down' during transport due to rough braking of the truck driver (falls) causing 3.8% of the total number of bruises. Shortly thereafter, it was observed that the downer animals were trampled and 'stamped' by other animals and could not stand up again. A fourth animal was stamped during lairage while it was lying on the floor.

The use of stick and the application of electric prod did not result in any bruise. From the number of bruises with known origin (n= 52), 26.9% resulted as consequence of a human-animal bruising event, 26.9% from an animal-animal bruising event and 46.2% from an animal-facility bruising event. The risk of bruising due to animal-facility events (OR= 83.6; 95% CI: 39.6-176.4) or human-animal events (OR= 10.6; 95% CI: 5.0-22.7) was significantly (P<0.0001) higher than the risk for bruising due to animal-animal interactions.

The potential bruising events and whether or not they resulted in a bruise are presented in Table 5. Out of 161 potential human-animal bruising events 9.0% resulted in a bruise. Of the 1,575 potential animal-animal bruising events less than 1.0% resulted in a bruise. In contrast, of the 56 potential animal-facility bruising events 43.0% resulted in a bruise.

The highest number of bruises were inflicted by animal-facility events in the stunning box (n=19, 36.5%).

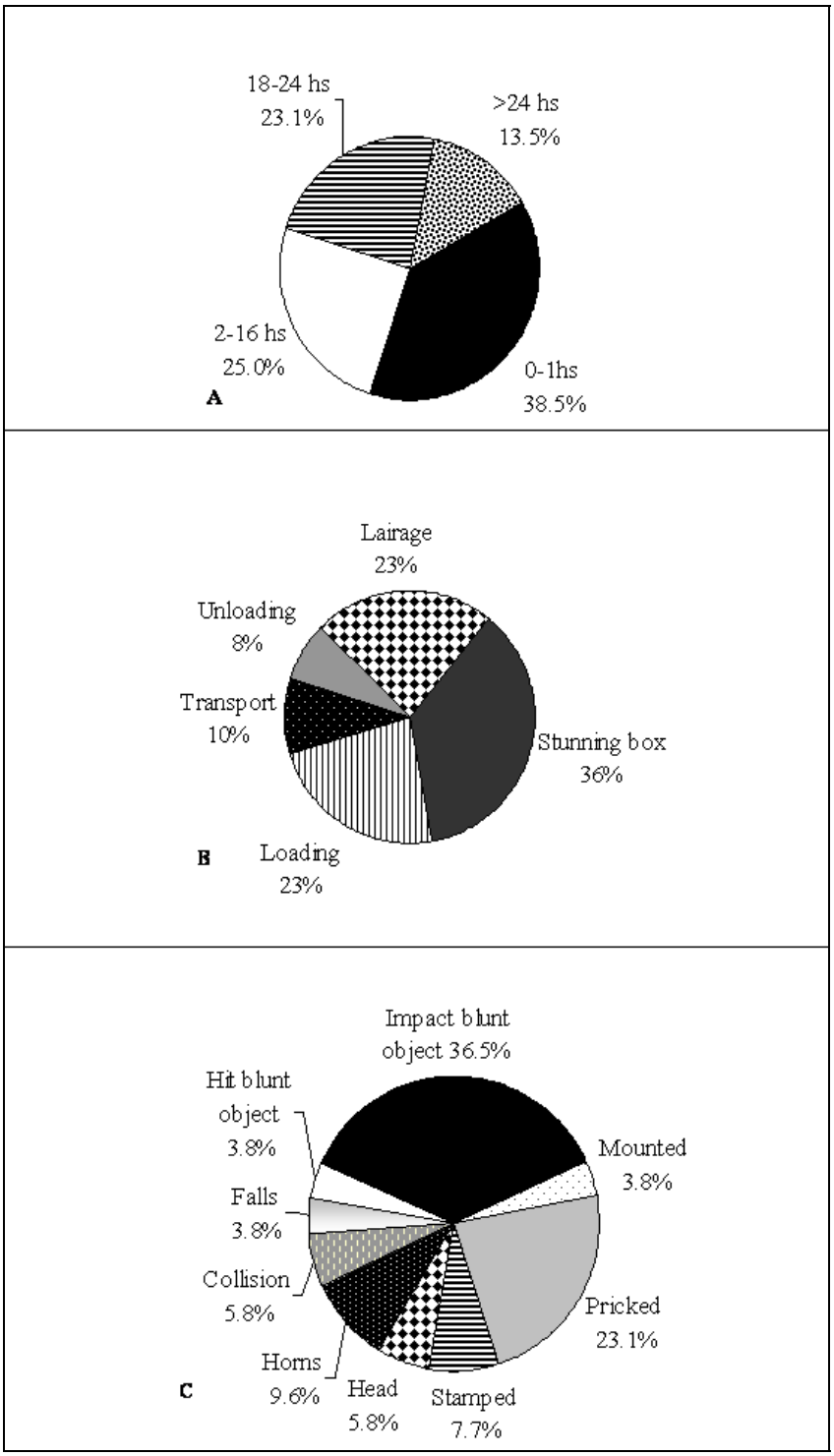

Figure 3. Pie charts showing 'when', 'where' and 'how' bruises were inflicted (n= 52 bruises on 37 cows)

(A) Time elapsed from loading at the farm until stunning.

(B) Pre-slaughter stage at which animals were at the moment of infliction

(C) Type of event which caused the bruise

Table 5. Type of bruising event according to the pre-slaughter stage where the animals were during the infliction of the bruises

Type of interaction		Total events observed	Events causing bruises n (%)					
			Loading	Transport	Unloading	Lairage	Stunning box	Total
Human animal	Hit with blunt object	28			2 (100.0)			2 (3.8)
	Hit with stick	40						0 (0.0)
	Poked with electric prod	55						0 (0.0)
	Pricked with sharp object	38	12 (100.0)					12 (23.1)
Animal Animal	Butted with head	1,281				3 (100.0)		3 (5.8)
	Butted with horns	26				5 (100.0)		5 (9.6)
	Stamped by other animal	4		3 (75.0)		1 (25.0)		4 (7.7)
	Mounted by a co-specific	264				2 (100.0)		2 (3.8)
Animal Facility	Impact with blunt object	47					19 (100.0)	19 (36.5)
	Collision	6			2 (66.7)	1 (25.00)		3 (5.8)
	Falls down	3		2 (100.0)				2 (3.8)
	Total	1,792	12 (23.1)	5 (9.6)	4 (7.7)	12 (23.1)	19 (36.5)	52 (100.0)

Distribution and visual appearance of the bruises and stage of infliction of the bruising event

Most of the bruises allocated on the back site were inflicted when the animal was in the stunning box (90.5%). The majority of the bruises found on the pin site (75.0%) were inflicted during loading at the farm. The anatomical site of the bruises was significantly associated with the stage at which the animal was at the moment of infliction (Fisher exact $P<0.0001$; chi-square= 88.7, df= 20).

At all stages most of the bruises inflicted were of grade 1. The severity grade was not significantly related to the pre-slaughter stage where the bruise was inflicted (Fisher exact *P*-value= 0.28; chi-square= 4.1, df= 4). Small bruises were most frequently inflicted in the stunning box (40.7%, n= 52) and at the farm during loading (37.0%); medium-sized bruises were mainly inflicted during lairage (43.8%); and large bruises -bigger than 16 cm- were mainly produced during transport (33.3%). The size of the bruises was significantly associated with the stage where bruises were sustained (Fisher exact *P*-value= 0.01; chi-square= 18.5, df= 8). Most of the bruises had irregular shape (92.3%) and were red coloured (61.5%); these characteristics of the bruises were not significantly associated with a pre-slaughter stage (Fisher exact *P*-values= 0.62 and 0.68; chi-squares= 6.1 and 2.5, df= 8 and 4, respectively).

Bruises with unknown cause (n= 26) were mainly grade 1 (92.3%), had small size (61.5%), with irregular-shape (100.0%) and 50.0% of them were red and 50.0% bluish coloured.

Discussion

The aim of this study was to assess the causal event of bruises during the pre-slaughter period in culled cows under commercial standard conditions in Chile. At *post mortem* evaluation a high proportion of bruised carcasses (71.5%) and also multiple bruises per carcass (average 2.1±1.2) were found. Video recording and direct observations analyses revealed that a high percentage of the bruises (46.2%) was caused by the impact of the animal with a metallic gate -'guillotine-type'- present in the stunning box. This vertical gate had a bottom edge without protection and impacted the animals on their back area when the box was closed. However, it should be considered that this was not an automatic gate since it was operated manually by a person. Therefore, training of the operators (María, 2008; Gallo, 2009) as well as

improvements in the condition of the facilities (Grandin, 2000) can contribute to decrease the risk of bruises.

Rough handling increases the incidence of bruises on carcasses (Nanni Costa *et al.* 2006). Our data is in line with these findings. It was observed that during loading of the animals at the farm, stock persons placed themselves behind the animals trying to move cattle quickly. Wooden sticks were frequently used to hit and also to poke (especially reluctant) cows. This rough handling caused 26.9% of all bruises and all of them were located on the pin site. There is a wide variety of animal-friendly devices available such as flags, flappers and bags which can be used to move cattle and to make them move forward (OIE, 2011). Even sticks can be used in a friendly way as an extension of the arms to move cattle without causing physical damage.

At first glance it appears that during loading at the farm the animals faced a lower number of bruising events (45) compared to lairage (1647) at the slaughterhouse. However, when the total number of bruising events was expressed per minute, the number of events that occurred during loading was higher (4.1 events/minute) than for lairage (1.4 events/minute). Although a high number of animal-animal interactions (mounting and butting) was observed during lairage, this was not proportional to the number of bruises that resulted from those events (14 bruises from 1,575 events). Kenny and Tarrant (1987) reported that the use of an overhead electrified wire grid in lairage prevented the mounting behaviour in re-grouped animals and this resulted in a significant reduction of carcass bruising. Also Warriss (1990) and Blackshaw et al. (1987) reported that mounting and aggressive interactions increase the risk for bruising. Our data did not concur with these findings. The difference may be explained by the fact that cows kept in the same pen were already familiar with each other and in consequence physical contacts are expected to be less severe. However, prolonged lairage time likely increase the opportunity of animal-animal interactions and the subsequent occurrence of bruises and therefore should be avoided (Gallo, 2009).

In the present study most of the bruises were observed on the back and pin sites of the carcasses (55.1%). Grandin (2000) reported that it is feasible to predict the bruise-inducing event observing the distribution of the bruises on the carcass. Our data are in this line since the bruises located on the back of the animal were caused by

equipment problems -and also due to the performance of the operator of the equipment- such as collisions with structures whilst bruises present on the pin were typically inflicted by rough handling during loading at the farm. However, it was not possible to associate 33.3% of the bruises with a bruising event.

Bruises can be a source of pain (Gregory 2004) and fear for the animals (Grandin, 2000). In welfare assessment, pain and sources 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). Ekesbo (1981) hypothesized that the status of the integument might offer a rapid tool to identify and evaluate the welfare of production animals and this study provides evidence of it.

Conclusion

We identified areas of most risk for bruising during the pre-slaughter stages of cows. These included rough handling due to inappropriate use of aids to drive animals during loading and unloading, and inadequate stunning facilities at the slaughterhouse. Improvements in these areas via designing appropriate structures and training of people for the handling of cattle can reduce the occurrence of bruises and in consequence lead to better welfare conditions of cattle for slaughter.

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Chapter 7

General Discussion

Introduction

Chile is a country that aims to improve beef production and meat quality on a short term (Foreign Investment Committee, 2010). Because of its sanitary status – e.g. free of Foot and Mouth Disease and Bovine Spongiform Encephalopathy – Chile has access to the most demanding markets (e.g. EU, USA, Japan) which pay higher prices compared to the domestic market (Valenzuela Fernandez et al., 2005). However, meat quality is affected negatively by the presence of bruises on the carcasses which causes economic losses. Bruised carcasses cannot be exported and the trimming of edible parts leads to downgrading of the carcass value (Gallo, 2009).

Consumers of export markets are also concerned about the ethical quality of meat, which is defined by the way animals are bred, raised, handled and slaughtered (Warriss, 2010). This is another aspect of meat quality that Chile has to comply with for entering the economically attractive markets. The presence of bruises affects the ethical quality of meat negatively since they are indicators of suboptimal welfare conditions during the pre-slaughter period.

Nowadays, only Chilean cattle transporters are held responsible for the economic losses due to bruising and the final grading of the carcasses is taken into account when the transport costs are calculated (Gallo, 2008). More information regarding the level of carcass bruising and their causes is needed to decide who is accountable for the losses on one hand, and on the other hand to take prevention measures to reduce carcass bruising and to improve the welfare of the animals.

The aim of this thesis was to study the occurrence, variation in characteristics and causes of bruises under Chilean conditions –from loading until slaughter- and to elucidate their relationship with the pre-slaughter conditions.

In this general discussion, the most important results as described in the previous chapters are evaluated and the main scientific and practical implications are discussed.

The prevalence of bruises in Chilean cattle

In Chapter 3, the prevalence of bruises in two slaughterhouses was determined, while using the records of 130,000 carcasses over one year, resulting in 8.6% and 20.8%, respectively. In Chilean slaughterhouses, bruises on carcasses are recorded routinely by an official meat grader assessing severity according the type of tissues affected in

the injured area. When only subcutaneous tissue is affected the bruise is scored as grade 1; grade 2 is assigned when subcutaneous and muscular tissues are affected, whereas bruised subcutaneous and muscular tissue with presence of broken bones is scored as grade 3 (INN Chile , 2002).

The discrepancy in prevalence between both slaughterhouses was partly attributed to the differences in scoring bruises affecting subcutaneous tissue (grade 1). Probably the meat grader of the slaughterhouse (one per meat plant) with lower bruise prevalence focused on bruises with real economical impact (grades 2 and 3) whereas superficial bruises (grade 1) were neglected. Such differences between meat graders i.e. slaughterhouses need obviously attention when comparing results of slaughterhouses.

The prevalence reported in Chapter 3 largely contrasts with the high prevalence reported in other Latin American countries such as Brazil (84.3%) or Mexico (92.0%) where animals are slaughtered using similar procedures as seen in Chile (Table 1). It can be argued that carcasses are actually bruised more often in these countries or it may also be related to the quality or interpretation of the official bruise scoring protocol used in Chile, which could lead to an underestimation of bruise prevalence, especially when it is linked to economic penalties.

A new extended protocol for bruise scoring

The current Chilean scoring system (INN Chile, 2002) is based on visual inspection of the carcass where only the most severe bruise of the carcass is recorded, leading to an underestimation of the actual prevalence of bruises on carcasses. Bruises are indicators of impaired welfare, and their gross appearance can indicate their causes (Grandin, 2000). Therefore a more detailed protocol including the anatomical location and visual characteristics of the bruises –like size, shape, grade and colour - is required, to link the appearance of bruises with pre-slaughter conditions. A new protocol was developed by merging the original Chilean system (INN Chile, 2002) with the Australian Carcass Bruising Scoring System (Anderson and Horder, 1979). Colour and shape were not in these scoring systems, but were added to the new protocol as well (Table 2).

Table 1. Prevalence of bruises as reported from several Latin American countries

Country	Reported by	Number of carcasses	Prevalence %	Parameters included in the bruise grading system
Argentina	Ghezzi et al. (2008)	15,361	57.8	Number of bruises per carcass and per anatomical site. Per bruise: size and severity grade (grade 1, 2 and 3)
Brazil	Andrade et al. (2008)	121	84.3	Number of bruises per carcass and per commercial cut
México	Miranda et al. (2011)	1,236	92.0	Number of carcasses with bruises. Per bruise, anatomical area, size and severity grade (grade 0, 1, 2 and 3)
Uruguay	Huertas et al. (2010)	15,168	60.0	Number of bruises per carcass
Chile	Godoy et al. (1986)	4,517	9.3	Presence of bruises on the carcass. Only the most severe bruise of the carcass is recorded. Severity grade (grade 1, 2 and 3)
	Gallo et al. (1999)	114,666	7.7	Idem
	Strappini et al. (2010)	127,838	12.3	Idem

In Chapter 5, the new protocol was used to make an inventory of the visual appearance of bruises of slaughtered animals coming from different sources. Outcomes showed that with this protocol variation in number of bruises per carcass and variation between bruises (grade, size, shape and colour), could be assessed which is a pre-requisite for studies evaluating the impact of bruises on welfare.

Table 2. Information collected using the current Chilean protocol and the new protocol.

Apply for	Current Chilean protocol	New extended protocol
All carcasses	Presence/absence of bruises	Presence/absence of bruises
Only for bruised carcasses	Grade (1, 2 or 3) of the most severe bruise	Severity grade of each bruise (grade 1, 2 or 3) Anatomical distribution of the bruises over the carcass (1, 2, 3, 4, 5, 6 or 7). Multiple-site bruises are scored Size of the bruise (small, medium or large) Shape (irregular, circular, lineal, tram-line, mottled) Colour (bright red, bluish, yellowish)

Besides the use of the new protocol by our observer, cow carcasses were scored by the official meat grader of the slaughterhouse as part of his daily routine. A large discrepancy appeared when both scores are compared: 92.0% of bruised carcasses in the first case and only 10.2 % in the second (Table 3). The current Chilean system does not differentiate between carcasses having one or multiple bruises. For animal welfare research, the number of bruises reflects the level of harm suffered by the animal in the *ante-mortem* period (Grandin, 2000). Therefore, the current Chilean system is subject to main constraints to identify animal welfare problems.

Table 3. Bruise scoring results from the current Chilean system and the new protocol (n = 264)

Scores		Chilean bruise grading system*	New bruise grading system**
Carcasses with bruises		9 (3.4%)	243 (92.0%)
Carcasses without bruises		255 (96.6%)	21 (8.0%)
Number of bruises/carcass	1	9 (100.0%)	36 (4.3%)
	2	-	86 (10.2%)
	3	-	150 (17.3%)
	4	-	171 (20.2%)
	5	-	155 (18.3%)
	6	-	132 (15.6%)
	7	-	84 (9.9%)
	8	-	24 (2.8%)
	9	-	18 (2.1%)
Total number of bruises scored		9 (100.0%)	846 (100.0%)

*Data courtesy slaughterhouse Carnes del Sur

**Data from Chapter 5

Reliability of the new bruise scoring system

Since the new bruise assessment protocol is based on 'subjective' scores, it was first investigated whether individual observers were consistent in carrying out the protocol and whether there was also agreement between observers in the assessment of bruises.

The reliability of scoring bruises using the new protocol appeared to be highly consistent within raters, i.e. when a rater scores the same bruises repeatedly (Chapter 4). Intra-observer reliability for the number of bruises per carcass was determined by means of calculating the ICC for three trained observers. Outcomes showed high intra-class correlation coefficients (ICC), varying from 'moderate agreement' (ICC= 0.78) to 'almost perfect agreement' (ICC= 0.95 and 0.91). Inter-observer reliability for the number of bruises was assessed using 4 observers and showed lower consistency, from 'fair agreement' (ICC= 0.43) to 'moderate agreement' (ICC= 0.80).

Based on outcomes of Chapter 4 one observer with good reliability scores was selected for the subsequent study on differences in number and characteristics of bruises of cows arriving to the slaughterhouse directly from farm or via markets (Chapter 5).

Type and source of the slaughtered animal and their relation with bruises

The literature reviewed in Chapter 2 indicates that the prevalence of bruising varies between types of slaughtered animals (i.e. cow, ox, heifer, steer, bull, calf). In Chapter 3 the presence of bruises was related to animal characteristics using data of one year of two Chilean abattoirs. This study showed that old animals -with 8 permanent incisive teeth- had a higher risk for bruising (cows, OR= 2.6 and oxen, OR= 2.2) than steers (OR= 1.0). This result is in line with Wythes et al. (1985) and McNally and Warriss (1996) who showed that cows had the highest bruise score of all cattle. The reason could be that cows are treated with less care due to their lower commercial value compared with other types of animals (Grandin, 2000). According to the OIE (2010), old animals require special conditions in the design of facilities and vehicles and additional attention during transport, however these recommendations are not considered in the current Chilean regulations (Chile MINAGRI, 2002).

The association between the method of selling (livestock market, livestock dealer or off farm) and the presence of bruises was also analyzed in Chapter 3. The

results showed that animals traded through a livestock market had increased risk for bruises (OR= 1.3) compared to animals that come from livestock dealers (OR= 1.0) or are transported directly from the farm (OR= 0.9). In addition, outcomes described in Chapter 5 showed that the method of selling was significantly associated with the number of bruises on the carcass (P<0.001). McNally and Warriss (1996) reported similar results. Carcasses of animals coming through markets were more often bruised (7.8%) than carcasses arriving from dealers (6.3%) or directly from farms (4.8%) (*P*<0.001).

As "cows" were the cattle category with the highest risk for bruising, we used this type of animals for the studies described in Chapters 5 and 6.

In Chapter 5, we compared the presence of bruises in cows transported directly from the farm with cows traded via a livestock market. In this study we found that marketed animals had more often bruised carcasses (95.2%) than animals from farms (88.3%) and also significantly (p<0.001) more number of bruises per carcass (*versus* 3.8±2.0 bruises/carcass from livestock markets *versus* 2.5±1.8 bruises/carcass from farm). These results are in line with those reported by Jarvis et al. (1995) and Weeks et al. (2002) who found that 71.0% of the animals that had passed through a market showed a bruised carcass compared to 53.7% of the animals from farms.

Given that bruises are indicators of animal welfare, it can be stated that welfare of cows sold through livestock markets is poorer compared with cows transported directly from farms. The more an animal is handled, the larger the probability of showing bruises on the carcass; suggesting that cows should be handled with extra care, especially when sold through livestock markets. In Chile, large numbers of animals (in total 953,286 cattle in 2009) are still sold through livestock markets (ODEPA, 2011) and most of them are send to slaughter. Therefore large progress in ethical quality of meat can be made when marketed animals would present less bruises when slaughtered.

Animal handling and its relation with bruises

This thesis presents evidence of rough handling and improper use of driving aids such as sticks. According to Weeks et al. (2002) bruises caused by the impact with sticks present a typical pattern composed by two parallel red marks, the so-called "tram-line" bruises. In Chapter 5, "tram-line" bruises were recorded on carcasses of marketed animals on the rib area. McNally and Warriss (1997) found similarly a

positive correlation between bruising and 'tram-line' bruises in market cattle when a stick was applied with force.

The OIE (2010) recommended to avoid painful procedures such as whipping or use of electric prods, large sticks and sticks with sharp ends to move animals. In Chile, the use of sticks with nails on top is forbidden (Chile MINAGRI, 2002). However, the use of sticks is allowed and it is still common that animal handlers use them inappropriately to poke cattle on the pin area (de Vries, 2011). Data presented in Chapter 6 are in line with this finding and show that 26.9% of the bruises were located on the pin site. Video recordings and direct observation showed that most of the bruises found on this area (75.0%) were inflicted during loading at the farm and were caused by a prick with a sharp object.

Current Chilean legislation does not provide enough protection to animals regarding animal handling largely because there is not enough enforcement of the existing legislation. Training of stock persons at all the stages of the meat chain (at farm, livestock markets, livestock transport and slaughterhouses) is the best way to improve the welfare of the animals.

Lairage time and its relation with bruises

In Chapter 3 it was reported that the risk of showing bruises was significantly lower ($P<0.001$) for cows and steers that spent over 12 hours in lairage compared to animals kept between 6 to 12 hours. Similar results were found in calves by Mach et al. (2007) who reported a lower frequency of trimmed meat on the carcass of animals that spent more than 11 hours in lairage compared with those that spent shorter time (3.61% vs. 1.77%). However, our data showed that oxen had a higher risk to present bruises when they were kept between 12 and 18 hours in lairage. It is unclear why some cattle type that are kept in lairage for longer periods of time show the lowest risk for bruises.

Outcomes of Chapter 6 show that a high number of interactions between animals occurred during lairage at the slaughterhouse. However, this high number of animal-animal interactions (mounting and butting) resulted in a relatively low number of bruises (only 14 bruises from 1,575 animal-animal events). In our study, animals from the same batch were familiar with each other. However, results could be different when within batches unfamiliar animals are kept together for a prolonged period of time. When unfamiliar animals are mixed, fighting and physical attacks can

increase to re-establish the social dominance relationships and leading to extra carcass bruising (Mench et al., 1990; Ndou et al., 2011). According to Gallo (2010) prolonged lairage time likely increases the opportunity for forceful animal-animal interactions and the subsequent occurrence of bruises and therefore lairage time should be minimized.

Cause of bruises

Chapter 6 deals with the moment of infliction of bruises, at which stage and due to which events - from onset of transport to slaughter - bruises are caused in culled cows. Most of the cows (71%) presented bruises and the majority of the bruises (67.3 %) occurred at the slaughterhouse. The impact of the animal with a blunt object caused 36.5% of all bruises, and rough handling contributed with 23.1%, whilst 9.6% of the bruises were caused by interactions between animals. Similar findings were reported in bulls by Nanni Costa et al. (2006) who found a relationship between the frequency of rough handling and inadequate facilities with the incidence of bruises on the carcasses.

It is worth mentioning that analyses reported in Chapter 6 corresponded to cattle transported directly from farm to slaughterhouse. According data described in Chapter 5 the percentage of bruised carcasses differs significantly if animals are transported directly from farm (88.3%) or from a livestock market and from there to the slaughterhouse (95.2%). This difference might be attributed to the extra loading, transport and unloading events that marketed animals have to overcome. Loading events contributed with 23.1% of the bruises, transport with 9.6%, whilst unloading with 7.7% of the total number of bruises (Chapter 6).

However, we found that the inadequate stunning facilities at the slaughterhouse and the rough handling of the stock people were the main causes of bruises. Since 36.5% of all bruises was caused by the impact of the animals with a metal gate of the stunning box, an improvement of the condition of the gate can include the attachment of soft-rubber protection. This simple and inexpensive measure would lead to a reduction of bruised carcasses by 1/3.

To improve both meat quality and welfare of slaughtered animals, simple and affordable measures can be taken which will be beneficial for both farmers and animals.

Research approach of this thesis

Data of all the studies of this thesis were collected under practical conditions. Inter-observer reliability assessment (Chapter 4), carcass evaluation (Chapter 5) and visual characterization of the bruises (Chapter 6) were all carried out at the same slaughterhouse. For the study described in Chapter 6, animals were supplied by three local farms and delivered to the slaughterhouse under routine practical conditions. For this thesis the approach of using real-life data was chosen for two reasons:

1. Applicability of the results: results of this thesis can be straightforwardly translated into commercial practice. Moreover, line speed, lightening, working-load, correspond to standard practices at an average commercial meat plant in Chile, and they would have been factors difficult to reproduce under experimental research conditions.

2. Ethical concerns: one of the original aims of the study was to relate the age of a bruise with colour and histochemical properties. The on-purpose infliction of bruises on experimental animals would allow us to know their age precisely. However, it would be ethically unacceptable to create bruises 'artificially', because bruises are a source of pain and distress for animals (Broom, 1998; Gregory, 2007). A bruise is a painful injury for two reasons. Firstly, the impact which leads to the bruise can be painful itself, because it produces a physical compression sufficient to disrupt blood vessels; and secondly, the subsequent swelling and inflammation at the site of the injury leads to a long lasting pain and sensitivity to pressure (Basbaum and Woolf, 1999). Consequently, the on-purpose infliction of bruises would have been harmful for the welfare of experimental animals and therefore, it was ruled out. Alternatively, it was chosen to perform observational studies to assess the potential moment of infliction of a bruise and to evaluate the bruises *post mortem* at the slaughter line.

Conclusions

This thesis shows the occurrence and variation of bruises and their relationship with the pre-slaughter conditions on Chilean cattle carcasses. It can be concluded that:

1. The accurate detection of bruises on the basis of a detailed protocol enables the identification and evaluation of sub-optimal welfare conditions for the animals during the pre-slaughter period. The current Chilean scoring system presents several

constraints to evaluate carcasses in this respect. The new protocol presented in this thesis has a much greater potential to be used in a monitoring system and to find measures that can be taken to prevent bruises.

2. When using the new protocol, the agreement between observers is low. However reliability seems sufficiently high when one observer performs the bruise evaluation.
3. Carcasses of old stock cattle (i.e. cows and oxen) have the highest risk to present bruises. This is aggravated when the animals pass a livestock market and subsequently are subjected to multiple loading, transport and unloading procedures.
4. Most bruises in cow carcasses are superficial, small, with irregular shape and red bright colour. This latter indicates that bruises are 'fresh', aging less than 24 hours and they are inflicted on the way from the farm to the slaughterhouse.
5. The distribution of bruises over the carcass is associated with the source of the animal.
6. Most of the bruises are caused by rough handling of the animals during loading at the farm and during unloading of the animals and by inadequate facilities at the slaughterhouse.

Recommendations to prevent bruises in cattle under Chilean conditions

Based on the results of this thesis the following recommendations to prevent bruises can be considered:

- Creation of beef quality assurance schemes to monitor the welfare of the animals along the meat chain. The scheme should set up minimal standards for animal welfare at each stage of the pre-slaughter period. The procedures to be inspected should cover the way animals are handled at loading at the farm, during transport, at unloading, during lairage and in the stunning box. Evaluation of facility conditions at the farm, livestock markets, transport and slaughterhouses should be included as well. Transport companies, and slaughterhouses that comply the standards will be considered for certification under the scheme. The Chilean meat industry should have special interest in this beef quality assurance scheme since consumers of export markets have an increased concern about how animals are treated and slaughtered in meat supplying countries.
- Reduction of the number of extra loadings, transports and unloadings of the animals by developing electronic livestock markets that bring livestock buyers, cattle and sellers virtually together.

- Training of stock persons on farm, at livestock markets and slaughterhouses in the principles of animal behaviour, welfare and handling of cattle using animal-friendly aids (e.g. flags, flappers, or plastic bags) to move animals. This point should be relatively easy and low-priced to incorporate into existing systems and may have positive effects on both, welfare of animals and on the quality of meat.

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Summary/ Samenvatting/ Resumen

Summary

Chile aims to become a 'food power country' in a short term and the improvement in the red meat industry is part of the agenda. Despite all the technological improvements that took place in the last years, losses in the way of meat trimmed and carcass downgrading due to bruising still continue.

Bruises are subcutaneous lesions that develop after application of force, which a) reduce meat quality and b) are indicators of poor welfare conditions. The latter is relevant due to increased concern of consumers in export markets about animal welfare. From a literature review (Chapter 2), a number of factors related to pre-slaughter conditions appeared to contribute to the presence of bruises in slaughtered cattle e.g. age and sex of the animal, transport conditions, method of selling through livestock auction markets, duration of lairage at slaughterhouses, rough handling of animals and some cattle characteristics, such as presence of horns and behaviour. For Chile, the contribution of each one of these factors to bruising is not clear.

The aim of this thesis was to study the relationship between pre-slaughter factors and characteristics and occurrence of bruises (from loading until slaughter) under Chilean conditions. To guarantee applicability of the results, all studies were conducted under practical conditions.

In the first study (Chapter 3), one year of slaughter records of two Chilean slaughterhouses were analysed regarding bruise scores and pre-slaughter conditions. Cows and oxen had higher risk to present bruises compared to steers and heifers, the younger animal categories. Moreover, animals that passed through a livestock market were more prone to present bruises than animals that came directly from the farm. Presence of bruises was significantly associated with increased carcass pH values. On the other hand, a large difference in carcass bruise prevalence (20.8 % versus 8.6%) was found between slaughterhouses. This discrepancy could be attributed to differences in the use of the Chilean scoring system, where some meat graders focus more on deepest bruises with economical impact rather than on superficial bruises.

The current Chilean bruise scoring system is quite general and only gives information related to the most severe bruise on the carcass in terms of depth of the infliction. More detailed data on each bruise on a carcass would allow a better deduction of potential bruising events and the pre-slaughter welfare circumstances for

the animal. Therefore a new scoring system was developed (Chapter 4) and its reliability was assessed. Four board-certificated meat graders participated to estimate the inter-observer agreement by simultaneously evaluating 46 carcasses at the slaughter-line. Intra-observer reliability of the observations was assessed with three meat graders, each of whom blindly examined 50 photos of bovine carcasses in two sessions. Results of this study showed a higher agreement when only one observer performs the scoring (from 'substantial' to 'moderate') compared to the inter-observer agreement (from 'fair' to 'moderate'). Based on outcomes of Chapter 4 one observer with good reliability scores was selected for the subsequent studies.

In Chile, a large number of animals are still sold via livestock markets, which involves multiple loading, transport, un-loadings and often mixing of unfamiliar animals. To investigate the characteristics and number of bruises on cattle carcasses and to relate these parameters to the source of the animals (directly transported from farm to slaughterhouse, or passing through a livestock market first) a new study was conducted (Chapter 5). For a total of 258 cow carcasses (111 from farm and 147 from market) the number of bruises, anatomical site, size, colour and shape was assessed, using the new protocol. Results showed that both group of cows, from farm or from livestock market, presented a high probability of having bruises (95.2% and 88.3% respectively). The number of bruises per carcass was higher in animals from markets than in off farm animals (mean 3.8±2.0 versus mean 2.5±1.8, respectively). Tram-line bruises that are evidence of impact with sticks, were found in marketed animals only. The distribution of bruises varied between sources. While animals from farms presented more bruises on the back site, animals from markets showed more bruises on the pin, hip and ribs site. The variation in bruise characteristics in animals with different origin may be associated with quality of the human-animal relationship, multiple journeys, extra (un)loadings and extra animal handling. It was concluded that the gross characteristics of the bruises are a valuable tool to identify and evaluate potentially some sub-optimal welfare conditions during the pre-slaughter period. To gain more information about the precise causes of the bruises the next study was carried out.

In the last study (Chapter 6), the causal event of bruises during the pre-slaughter period was assessed. Fifty two culled cows were transported to the slaughterhouse in three batches under similar transport conditions. A combination of direct observation and video analyses was used to determine moment, pre-slaughter

stage and cause of potential bruising events during the period from loading on the farm till stunning at the slaughterhouse. During the *post mortem* evaluation of the carcass, the actual number of bruises, anatomical location on the carcass and characteristics of the bruises were established, using the new protocol. The results of this study showed that most bruises were caused by interactions between animal and facility structures, and most of them were inflicted in the stunning box at the slaughterhouse and appeared on the back site of the carcass. Bruises on the pin area were mostly caused during loading at the farm when animals were poked with a sharp object.

This thesis shows that the use of a detailed bruise scoring protocol allows the identification of the sub-optimal welfare conditions during the pre-slaughter period. This is relevant in order to take measures to prevent bruises.

Regarding the effect of the source of the animals on the number of bruises on the carcass, results confirm that animals passing through a livestock market have more bruises than animals transported directly from the farm to the slaughterhouse. Moreover, this thesis presents evidences of rough handling and animals beaten by sticks at markets. Carcasses of old stock cattle (i.e. cows and oxen) have the highest risk to present bruises. Therefore, old animals should be transported with extra care, and preferably directly from the farm to the slaughterhouse.

In conclusion, rough handling due to inappropriate use of aids to drive animals during loading and unloading, and inadequate stunning facilities at the slaughterhouse were the areas of most risk for bruising. Improvements in the design and maintenance of appropriate structures (e.g. at the farm, for transport, on markets, at slaughterhouses) and training of stock people for the handling of cattle will reduce the occurrence of bruises and in consequence will lead to better welfare conditions of cattle for slaughter.

Samenvatting

Het is Chili's doelstelling om op korte termijn een 'wereldvoedselmogendheid' te worden en het verbeteren van de (rood) vleesindustrie is onderdeel van de geplande veranderingen. Ondanks de in de afgelopen jaren doorgevoerde technologische verbeteringen, treedt er nog steeds veel verlies van vlees door kneuzingen (blauwe plekken) in het vlees. Dit omdat kneuzingen worden weg gesneden van het vlees en de karkaswaarde door kneuzingen in het vlees verminderd.

Kneuzingen zijn onderhuidse laesies die ontstaan nadat er druk op het weefsel is uitgeoefend. Deze verminderen vleeskwaliteit en zijn een indicator voor slechte welzijnsomstandigheden. Dit laatste is van belang omdat consumenten in exportmarkten in toenemende mate bezorgd zijn om dierwelzijn. Uit literatuuronderzoek (Hoofdstuk 2) is gebleken dat verschillende factoren, die gerelateerd zijn aan de omstandigheden voor slachten, een bijdrage kunnen leveren aan het optreden van kneuzingen bij geslacht rundvee. Voorbeelden hiervan zijn leeftijd en geslacht van het dier, transportomstandigheden, verkoopmethode (wel of niet via veemarkten), duur van het opstallen in het slachthuis, ruwe omgang met de dieren en sommige diereigenschappen zoals het hebben van hoorns en individueel gedrag. Het is voor Chile niet duidelijk wat elke afzonderlijke factor bijdraagt aan het optreden van kneuzingen.

De doelstelling van dit proefschrift was om de relatie tussen de omstandigheden voor slacht en het optreden van kneuzingen (van laden tot slachten) in Chili te onderzoeken. Om de praktische toepasbaarheid van de bevindingen te waarborgen zijn alle onderzoeken in praktische omstandigheden uitgevoerd.

In het eerste experiment (Hoofdstuk 3) zijn de slachtgegevens van één jaar van 2 Chileense slachterijen geanalyseerd met betrekking tot kneuzingscores en omstandigheden voor het slachten. Hieruit bleek dat koeien en ossen een groter risico op kneuzingen hadden dan stieren en vaarzen, die in de jongere diercategorie vallen. Verder hadden dieren die via een veemarkt verhandeld werden een grotere kans om kneuzingen te hebben dan dieren die direct van de boerderij kwamen. Dieren zonder vetlaag hadden de minste kans op kneuzingen. Kneuzingen waren significant gecorreleerd met verhoogde karkas PH waarden. Aan de andere kant bestond er een groot verschil in de prevalentie van karkaskneuzingen (20.8 % tegen 8.6%) tussen de

2 slachterijen. Dit verschil kon worden verklaard door een verschillend gebruik van het Chileense scoringssysteem voor kneuzingen, omdat sommige vleeskeurmeesters meer focussen op diepe kneuzingen (met economische schade) dan op oppervlakkige kneuzingen.

Het huidige Chileense scoringssysteem is erg algemeen en verstrekt alleen informatie over de diepte van de ergste kneuzing op het karkas. Meer gedetailleerde informatie over elke karkaskneuzing op het karkas zou kunnen leiden tot een beter inzicht in potentiële kneuzing veroorzakende omstandigheden en daarmee van de welzijnsomstandigheden voor slachten. Daarom is er een nieuw scoringssysteem ontwikkeld (Hoofdstuk 4) en op betrouwbaarheid getest. Vier gecertificeerde vleeskeurmeesters participeerden in het schatten van de inter-observator variatie door simultaan 46 karkassen aan de slachtlijn te beoordelen. De intra-observator betrouwbaarheid werd in kaart gebracht met 3 vleeskeurmeesters die elk 50 foto's van runderkarkassen onderzochten in 2 sessies. Uit dit onderzoek is gebleken dat er een grotere samenhang van scores is als alleen 1 keurmeester de karkassen beoordeelt (van 'substantieel' tot 'gemiddeld') dan wanneer dit wordt vergeleken met de tussen observator beoordeling (van 'gemiddeld' tot 'redelijk'). Gebaseerd op de uitkomsten van Hoofdstuk 4 is er 1 vleeskeurmeester met goede scoringsbetrouwbaarheid geselecteerd voor de vervolgstudies.

In Chili wordt een grote groep dieren nog steeds verkocht via veemarkten, dit impliceert meerdere malen laden, vervoeren, uitladen en vaak het samenvoegen van onbekende dieren. Om de karakteristieken van kneuzingen en het aantal kneuzingen op runderkarkassen te onderzoeken en dit te kunnen relateren aan waar de dieren vandaan komen (direct vervoerd van de boerderij of via een veemarkt) is er een nieuw experiment uitgevoerd (Hoofdstuk 5). Van in totaal 258 koekarkassen (111 van de boerderij en 147 van de veemarkt) is het aantal kneuzingen en locatie op karkas, grootte, kleur en vorm van de kneuzingen onderzocht met behulp van het nieuwe scoringssysteem. Beide groepen dieren (van boerderij en veemarkt) had meestal één of meer kneuzingen op het karkas (respectievelijk 95.2% en 88.3%). Het aantal kneuzingen per karkas was hoger voor dieren van de veemarkt dan voor dieren die van de boerderij kwamen (gemiddeld 3.8±2.0 tegen 2.5±1.8 kneuzingen). Tramlijn kneuzingen, die het resultaat zijn van stokslagen warden alleen in dieren van de veemarkt gevonden. De verdeling van de kneuzingen over het karkas was verschillend voor dieren van verschillende herkomst. Dieren die van de boerderij afkwamen

hadden meer kneuzingen op hun rug terwijl veemarktdieren meer kneuzingen hadden rond de staart, op de heupen en bij de ribben. Deze verschillen tussen dieren van verschillende herkomst is waarschijnlijk geassocieerd met de kwaliteit van mens-dier relatie, meervoudig transport, extra laden en lossen en extra diermanipulaties. De conclusie was dan ook dat het karakteriseren van kneuzingen waardevol is om suboptimale dierwelzijnsomstandigheden tijdens de periode voor slacht te identificeren en evalueren. Om meer te weten te komen over de precieze oorzaak van kneuzingen werd het volgende experiment uitgevoerd.

In het laatste hoofdstuk (Hoofdstuk 6), zijn mogelijke oorzaken van kneuzingen tijdens de periode voor slachten nader onderzocht. In totaal werden tweeënvijftig koeien getransporteerd naar het slachthuis in 3 batches met vergelijkbare transportomstandigheden. Een combinatie van directe observaties en video-opnames werd gebruikt om potentiele omstandigheden die een kneuzing zouden kunnen veroorzaken vast te stellen tijdens de periode van laden op de boerderij tot verdoven in het slachthuis. Tijdens het *post-mortem* onderzoek van de karkassen werden het aantal kneuzingen, de locatie op het karkas en de eigenschappen van de kneuzingen geanalyseerd met behulp van het nieuwe scoringssysteem. Op deze manier konden relaties worden vast gesteld tussen aard en type omstandigheden die aanleiding gaven tot een kneuzing zoals gevonden op het karkas. De resultaten van dit onderzoek wezen uit dat de meeste kneuzingen werden veroorzaakt door interacties tussen dier en structuren van het slachthuis en de meeste kneuzingen werden veroorzaakt in de verdovingsbox van het slachthuis en werden gevonden op de rugzijde van het karkas. Kneuzingen rond de staart werden voornamelijk veroorzaakt tijdens het laden van de dieren op de boerderij wanneer de dieren met een scherp voorwerp werden geprikt.

Dit proefschrift laat zien dat het gebruik van een gedetailleerd kneuzing scoringssysteem gebruikt kan worden om suboptimale dierwelzijnsomstandigheden tijdens de periode voor slachten te identificeren. Dit is belangrijk om maatregelen te nemen om kneuzingen te kunnen voorkomen.

De resultaten bevestigen dat de herkomst van de dieren een effect heeft op het aantal kneuzingen op een karkas. Dieren van de veemarkt hebben meer kneuzingen dan dieren die direct van de boerderij naar het slachthuis worden getransporteerd. Verder laat dit proefschrift bewijzen zien voor het ruw behandelen van dieren en het gebruik van stokslagen op de veemarkt. Karkassen van oudere dieren (bijv. koeien en

ossen) lopen het grootste risico om kneuzingen te hebben. Daarom zouden oudere dieren voorzichtiger moeten worden vervoerd en het verdient de voorkeur om deze direct van boerderij naar het slachthuis te vervoeren.

Dit alles leidt tot de conclusie dat ruw behandelen door onjuist gebruik van hulpmiddelen om de dieren op te drijven tijdens laden en lossen en ondermaatse verdovingsfaciliteiten in het slachthuis de grootste risicofactoren voor kneuzingen zijn. Verbeteringen in ontwerp en onderhoud van de juiste faciliteiten (op de boerderij, tijdens transport, op veemarkten en in slachthuizen) en training van veedrijvers/verzorgers zal het optreden van kneuzingen verminderen en welzijnsomstandigheden van slachtvee verbeteren.

Resumen

Chile es un país que aspira a convertirse en potencia agroalimentaria en corto tiempo y el mejoramiento del sector de carnes rojas es parte de la agenda. A pesar de todos los avances tecnológicos que se produjeron en los últimos años, las pérdidas por expurgos y las bajas de categoría en las canales debido a contusiones aún continúan.

Las contusiones son lesiones subcutáneas que se presentan después de la aplicación de una fuerza. Las contusiones a) reducen la calidad de la carne y b) son indicadores de pobres condiciones de bienestar. Este último punto es relevante debido a la creciente preocupación de los consumidores de los mercados exportadores por el bienestar animal. La revisión bibliográfica (Capitulo 2) muestra que hay una serie de factores relacionados con las condiciones previas a la faena del animal que contribuirían a la presencia de contusiones en el ganado. Entre ellos se citan la edad y el sexo del animal, las condiciones de transporte, la comercialización de de los animales a través de ferias ganaderas, el tiempo de espera en las plantas faenadoras de carne, el manejo rudo de los animales y algunas características propias de los animales tales como la presencia de cuernos y su comportamiento. En Chile, no es clara la contribución de cada uno de est os factores para ocasionar contusiones.

El objetivo de esta tesis fue estudiar la relación entre los factores previos a la faena, las características y ocurrencia de las contusiones (desde la carga hasta el sacrificio) bajo las condiciones de Chile. Para garantizar la aplicabilidad de los resultados, todos los estudios fueron llevados a cabo en condiciones comerciales.

En el primer estudio (Capitulo 3), lo registros anuales de dos plantas faenadoras de carne fueron analizados con respecto a la graduación por contusiones y a las condiciones previas al sacrifico. Las vacas y los bueyes tuvieron el riesgo más alto de presentar contusiones comparado con las categorías más jóvenes tales como las vaquillas y los novillos. Más aún, los animales que pasaron por una feria ganadera fueron más susceptibles de presentar contusiones que aquellos animales que venían directamente de la granja. La presencia de contusiones estuvo significativamente asociada con canales con altos valores de pH. Por otra parte, se encontró una gran diferencia en la prevalencia de contusiones entre distintas plantas faenadoras (20.8% versus 8.6%). Esta discrepancia en los valores podría ser atribuida a diferencias en el uso del sistema chileno de clasificación de contusiones, donde algunos certificadores

de carne concentraron sus registros en las contusiones más profundas con impacto económico más que en las contusiones superficiales.

El actual sistema de clasificación de contusiones es bastante general y sólo da información relacionada con la contusión más severa de la canal en términos de profundidad de la lesión. Información más detallada sobre las características de cada contusión de la canal permitiría deducir cuales son los potenciales eventos así como las circunstancias previas al sacrifico del animal que podrían haber causado la lesión. De este modo un nuevo sistema de clasificación de contusiones fue desarrollado (Capitulo 4) y su confiabilidad fue evaluada. La concordancia entre observadores fue evaluada con cuatro certificadores oficiales quienes clasificaron simultáneamente 46 canales bovinas directamente en la línea de faena. En tanto que la concordancia para cada observador fue determinada en tres certificadores de carne quienes evaluaron de manera ciega 50 fotos de canales bovinas durante dos sesiones. Los resultados de estos estudios muestran un alto grado de acuerdo cuando un solo observador realiza la evaluación (de sustancial a moderado acuerdo) comparado con la concordancia entre observadores (de regular a moderada). Basada en los resultados del capitulo 4, un solo observador con buen nivel de concordancia fue seleccionado para realizar los siguientes estudios.

Hoy en día en Chile, un gran número de animales son todavía vendidos a través de ferias ganaderas, lo cual implica múltiples cargas, transporte, descargas y a menudo la mezcla de animales desconocidos. Para investigar las características y el número de contusiones en las canales bovinas y relacionar estos parámetros con el origen de los animales (directamente transportados desde la granja o pasando previamente por una feria ganadera) un nuevo estudio fue llevado a cabo (capitulo 5). Para ello 258 canales de vacas fueron evaluadas (11 canales de animales de predio y 147 de animales de feria). El número de contusiones, la localización anatómica, su tamaño, color y forma fueron determinados usando el nuevo protocolo. Los resultados mostraron que ambos grupos de vacas, provenientes de predio o de feria, presentaban una alta probabilidad de tener contusiones (95.2% y 88.3% respectivamente). Sin embargo, el número de contusiones por canal fue significativamente mayor en los animales de feria que en los animales de predio (promedio 3.8±2.0 versus 2.5±1.8 respectivamente).

La forma de una contusión puede indicar el objeto que la causo. Así, por ejemplo, las contusiones con forma de “vías de tren” son evidencias del impacto con

palos y estas contusiones fueron encontradas solamente en animales de feria. La distribución de las contusiones en la canal varió de acuerdo al origen de los animales, mientras que animales provenientes de granja presentaban mas contusiones en la zona dorsal, animales de feria mostraron más contusiones en los glúteos, caderas, y costillas. La variación en las características de las contusiones en animales con distinto origen podría estar asociada con la calidad de la relación humano-animal, los múltiples transportes, las cargas y descargas adicionales así como al manejo extra de los animales. Se puede concluir que la descripción detallada de las características macroscópicas de las contusiones es una herramienta valiosa para identificar y evaluar condiciones potencialmente aversivas durante el periodo previo al sacrificio del animal. Para tener más información sobre las causas precisas de las contusiones se llevo a cabo un nuevo estudio.

En el último estudio (Capítulo 6) los eventos causales de las contusiones durante el transporte de los animales hacia la planta faenadora de carnes en tres lotes bajo similares condiciones. A través de una combinación de observaciones directas con análisis de videos se determinó el momento, el estadio ante mortem y la causa de los eventos que potencialmente podrían ocasionar contusiones desde la carga en predio hasta el momento de noqueo en la planta faenadora. Durante la evaluación post mortem de la canal, el número total de contusiones, su localización anatómica en la canal y las características de las mismas fue determinada usando el nuevo protocolo. Los resultados de este estudio muestran que la mayoría de las contusiones fueron causadas por las interacciones ente los animales con las instalaciones, y que la mayoría de ellas fueron infligidas en el cajón de noqueo apareciendo en la zona dorsal de las canales de los animales. Las contusiones presentes en los glúteos fueron principalmente causadas durante la carga en el predio cuando los animales fueron punzados con un objeto con punta afilada para forzarlos a moverse.

Esta tesis muestra que el uso de un protocolo detallado para evaluar las contusiones permite la identificación de las condiciones su-óptimas de bienestar animal durante el periodo ante mortem. Esto es relevante para tomar medidas para prevenir contusiones.

Con respecto al efecto del origen de los animales y el número de contusiones en la canal, los resultados confirman que los animales que pasan a través de una feria ganadera tienen mas contusiones que los animales transportados directamente del predio a la planta faenadora. Más aun, esta tesis presenta evidencias de trato rudo y

brusco hacia los animales y de golpes con palos en las ferias ganaderas. Las canales de animales viejos (ej. vacas y bueyes) tienen un riesgo mayor de presentar contusiones. Por lo tanto, los animales viejos deberían ser transportados con especial cuidado y preferentemente directamente del predio a la planta faenadora.

En conclusión, el manejo rudo de los animales debido al uso inapropiado de elementos de arreo durante la carga y la descarga, y las condiciones sub-óptimas del cajón de noqueo fueron las áreas de mayor riesgo para producir contusiones. Mejoras en el diseño y el mantenimiento de la infraestructura (ej. en el predio, transporte, en ferias ganaderas y en la planta faenadora) y el entrenamiento de los operarios en el manejo del ganado reducirá la ocurrencia de contusiones y en consecuencia llevará a un mejor condiciones de bienestar animal del ganado faenado.

List of publications

Refereed Scientific Journals

Strappini, A.C., Metz, J., Gallo, C., Kemp. 2009. Origin and assessment of bruises in beef cattle at slaughter. Animal 3: 5, 728-736

Strappini, A.C., Frankena, K., Metz, J., Gallo, C., Kemp, B. 2010. Prevalence and risk factors for bruises in Chilean bovine carcasses. Meat Science 86: 3, 859-864

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Strappini, A.C., Frankena, K., Metz, J.H.M., Kemp, B. 2012. Intra- and inter-observer reliability of the post mortem evaluation of bruises in Chilean beef carcasses. Livestock Science 145: 1, 271-274

Strappini, A.C., Metz, J.H.M., Gallo, C., Frankena, K. Vargas, R., de Freslon, I., Kemp, B. Bruises in Chilean culled cows: when, where and how are they inflicted? Animal (submitted)

Muñoz, D., Strappini, Gallo, C. Using animal behaviour and handling by personnel as animal welfare indicators during the stunning process of cattle. Archivos de Medicina Veterinaria (submitted)

Contribution in books

Strappini-Asteggiano, A.C. 2010. Problemas y errores más comunes encontrados en Chile durante el manejo del ganado. In: Bienestar Animal y Calidad de la Carne. (Eds.) Mota-Rojas, D. y Guerrero-Legarreta, I. Editorial BM Editores. Mexico.

Conference Proceedings and Abstracts

Strappini, A.C., Frankena, K., Metz, J. Gallo, C., Kemp, B. 2008. Presence of carcass bruises in slaughtered beef cattle in Chile. Proceedings 4th International Workshop on the assessment of animal welfare at farm and Group level. Ghent, Belgium (p. 149).

Strappini, A.C., Sandoval, M.L., Gil, H., Silva, R., Gallo, C. 2008. Utilization of a new protocol for beef carcass bruising evaluation. Proceedings XXXIII

Congreso de la Sociedad Chilena de Producción Animal. Valdivia, Chile (pp. 245-246).

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Strappini, A.C., Valenzuela, R., Navarro, G., Gallo, C. 2009. Contusiones en canales bovinas: cuantificación y caracterización macroscópica. I Encuentro Regional de Investigadores en Bienestar Animal, America. Valdivia, Chile

Valenzuela, R., Navarro, G., Strappini, A. and Gallo, C. 2009. Estudio comparativo de dos pautas de evaluación de contusiones en canales bovinas. I Encuentro Regional de Investigadores en Bienestar Animal, America. Valdivia, Chile

Estrada, G., Strappini, A.,Gallo, C. Comportamiento de novillos en corrales de espera en matadero posterior a transportes largos y cortos. I Encuentro Regional de Investigadores en Bienestar Animal, America. Valdivia, Chile

Strappini, A.C., Metz, J., Frankena, K., Gallo, C., Kemp, B. 2010. Bruise evaluation of cow carcasses arriving at the slaughterhouse from market or directly from the farm. Proceedings XXVI World Buiatric Congress. Santiago, Chile (p. 146)

Estrada Dávila, G., Strappini, Asteggiano A., Gallo Stegmaier. 2010. Efecto de la distancia de transporte sobre el comportamiento de novillos en corrales de espera durante el periodo previo a la faena. XIII Congreso Nacional y X Iberoamericano de Etologia. Castilla La Mancha, España (p. 146).

de Freslón, I., Strappini, A., Soto-Gamboa, M., Gallo, C. 2011. Reactividad de novillos durante el pesaje en romana y patrones conductuales. II Congreso Nacional de Etologia y II Encuentro de Psicología Comparada. Santiago, Chile

de Freslon Loisel, Strappini, A., Herrera, C., Gallo, C. 2011. Estudio preliminar sobre la respuesta conductual de novillos durante el pesaje en romana. II Congreso Latinoamericano de Etología Aplicada y Segunda Reunión Regional de la International Society for Applied Ethology America Latina. Ilhéus, Brasil.

Strappini, A. Las contusiones en bovinos como indicadores de bienestar: dónde, cuándo y cómo ocurren. 2012. II Encuentro Regional de Investigadores en Bienestar Animal: Avances y estrategias para el futuro de la producción pecuaria. Montevideo, Uruguay

Other Publications

Strappini, A.C.; Frankena, K.; Metz, J.H.M.; Gallo, C.; Kemp, B. 2009. Presence of bruising in cattle beef carcasses in Chile. WIAS Science Day.

Strappini, A. 2009. Lesiones y bienestar Animal. Workshop organized by Universidad de Chile, the World Society for Protection of Animals (WSPA) and Animal Health Service (SAG). Santiago, Chile

Strappini, A. 2009. Problemas de bienestar animal: transporte y sacrificio. Concepts in animal welfare. Workshop organized by the World Society for Protection of Animals (WSPA), Montevideo, Uruguay.

Strappini, A. El bienestar animal y el rol del medico veterinario. Master Class, Aula .Magna, Universidad Mayor. Temuco, Chile

Curriculum Vitae

Ana Carolina Strappini Asteggiano was born on August 4, 1966 in Rafaela, Argentina. After completing high school (Colegio Nuestra Señora de la Misericordia) in 1983 she studied Natural Science Teacher at Instituto Superior J.V. Gonzalez, Argentina. After graduation in 1988 she lectured Biology and Chemistry in several high schools and colleges. To specialize in Ethology Ana spent one year (1995-1996) at the Biology Institute of Humboldt University, in Berlin, Germany where she worked in a project entitled "Breeding behaviour of *Falco peregrinus* in Brandenburg". Back in Argentina she worked as ethology adviser at the NGO Habitat & Development Foundation in Santa Fe.

In 1999, she started the MSc in Animal Science at Wageningen University graduating in 2001, and her thesis titled "Physiological correlates of abnormal oral behaviour in veal calves" was oriented to Ethology. After graduation Ana worked at the Institute of Agricultural and Environmental Engineering (former IMAG-DLO) in Wageningen in a number of projects related to ethology and animal welfare of dairy cows and laying hens.

In 2005 she moved with her family to Chile, where she worked in the project "Diagnosis of the situation and development of strategies to improve animal welfare and meat quality in ruminants for human consumption". In 2007, Ana enrolled as PhD candidate at Adaptation Physiology Group, Wageningen University. Same year she was pointed out as Adjunct Professor *ad-honorem* at Animal Science Institute of Universidad Austral de Chile in Valdivia, and in 2011 she started also lecturing Ethology and Animal Welfare at Universidad Mayor in Temuco.

Ana is the proud mother of two daughters, Melisa (17) and Francesca (9). Since January 2012 she works for Veterinarian Without Borders-Canada (VWB), Latin-American office.

Acknowledgements

Soon after moving from The Netherlands to Chile I joined a project on assessment of animal welfare in slaughtered animals and then I was offered to do a PhD. A "Sandwich Program" in Wageningen looked suitable for me and I enrolled in that program. Because of what it meant to me to travel overseas frequently, and to live between two different worlds. In the way, I met many wonderful people who stood by my side during the best and worst moments of my thesis. Today I would like to express my gratitude to them.

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Special thanks go to my two paranymphs (actually three!), Jessika van Leeuwen and Mariëlle Bruijnis. Jessika, we met during the first course we attended during our PhD program: 'WIAS, Introduction course'. I still remember my confusion when I saw you: a blond Dutch-looking girl with a thermo under her arm drinking 'mate' (traditional Argentine infusion)!… Since that day we became good friends. Our conversations about Argentine people, PhD difficulties, frustration and enthusiasm were unforgettable. I wish you and Juan a lot of joy and happiness with your daughter! Mariëlle, we share office and the love for cows. Thanks for long

conversations after lunches and friendship. Nice that my paranymphs wanted to be and success with the completion of your dissertation!

I would like to thank my ADP office-mates for their camaraderie: Elske, Inonge, Marianna, Irene and Nanda who made me feel at home, all of you provided me a friendly environment in which I could work every time I was at Zodiac.

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Special thanks go to my colleagues and students of the Animal Welfare Group of Universidad Austral de Chile, who composed the 'bruises team': Ronald Vargas, Inés de Freslon, Grisel Navarro, Ricardo Valenzuela, Graciela Estrada, Juan Tarumán and Romina Concha. They spent many hours at the slaughterhouse scoring carcass bruises always in a good environment. You guys were indispensable in the execution of the studies of this thesis! Thank you also to Diego Muñoz, Luis Carter, Carla Herrera, Carlos Muñoz, Maaike de Vries and Gonja Witteveen for your help and contribution.

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My time at Wageningen was made enjoyable in large part due to the many Dutch and Latin friends that became a part of my life: Vicky and Herman, Nancy and Sam, Carmen and Rene, Lucilla and Taeke, Shirley and Sitze, Sonia and Huub, Clara, Greys, Arantza, Irene, Quela, Fanny, Tere and Nico, Tatiana, Daniela. You help me to contain emotions, joys, frustrations and concerns, always with patience and love. Gracias amigos por los momentos compartidos!

I would like to thank my new colleagues of Veterinarian Without Borders-Canada, Elena Garde and Guillermo Perez. Thanks for your support during the last stages of my thesis. You guys showed me that is possible to use science to improve the quality of life of animals and people. Your work is amazing!

I am more than lucky having the family that I have. Gustavo, thanks for your endless love. You were by my side whenever I lost courage and you always believed that I would finish my PhD. Thanks for being such 'atypical latin husband': to spent a couple of months taking care of our daughters when I was abroad, was not a problem for you. You are a perfect soul-mate! Te quiero mucho!!

Melisa and Francesca thank you for being positive and joyful daughters; you were a powerful source of inspiration and energy during difficult days…Chicas, las amo!! I'm looking forward to spend summer holidays together with you and papa!

Finally, I would like to dedicate this work to my lost parents, Ricardo and Leiter who left us too soon. I miss you…hope that this work makes you proud.

Ana

Training and Supervision Plan

Description and credits	Year
The Basic Package (3.0 ECTS)	
WIAS Introduction Course	2007
Course on Philosophy of Science and/or Ethics	2007
International conferences (4.6 ECTS)	
WAFL, 4th International Workshop. Ghent, Belgium	2008
Animal welfare in production animals. Montevideo, Uruguay	2008
Knowing animals. Florence, Italy	2009
1st International meeting of researchers on animal welfare. Valdivia, Chile	2009
XXVI World Buiatrics Congress. Santiago, Chile	2010
Seminars and workshops (4.7 ECTS)	
Workshop on animal welfare in slaughterhouses. Valdivia, Chile	2007
Concepts in Animal Welfare, WSPA. Valdivia, Chile	2007
International seminar on animal welfare in dairy cattle. Valdivia, Chile.	2008-2010
WIAS Science day. Wageningen, The Netherlands	2007-2010
Animal welfare in intensive production systems. Santiago, Chile	2010
International workshop on livestock transport. Chile-UE. Valdivia. Chile	2011
Presentations (10.0 ECTS)	
Workshop on animal handling during transport. Valdivia, Chile (Oral)	2007
WAFL, Ghent, Belgium (Poster)	2008
Concepts in Animal Welfare. WSPA. Montevideo, Uruguay (Oral)	2008
WIAS Science Day. Wageningen, The Netherlands (Poster)	2008
SOCHIPA, Chilean Society of Animal Production (Oral)	2007-2008
International meeting of researchers on animal welfare. Valdivia, Chile (Poster)	2009
XXVI World Buiatrics Congress. Santiago, Chile (Oral)	2010
XIII National Congress of Ethology. Ciudad Real, Spain (Poster).	2010
Scientific meeting of the Faculty of Veterinary Medicine (UACh) Valdivia, Chile (Oral)	2011
In-Depth Studies (6.2 ECTS)	
Farm Animal Welfare. Mediterranean Agronomic Institute of Zaragoza, Spain	2007
Humanitarian slaughter of bovines, porcine and poultry. WSPA. Valdivia, Chile	2011
Design of Animal Experiments. Wageningen, The Netherlands	2008
Veterinary Meat Inspection (Partial Time Attendance). Valdivia, Chile.	2012
Professional Skills Support Courses (5.4 ECTS)	
Scientific publishing, Wageningen, The Netherlands	2007
Techniques for scientific writing and presenting a scientific paper.	2007
Information literacy PhD including Endnote introduction, Wageningen.	2008
Scientific writing. Wageningen, The Netherlands	2010
Project and time management. Wageningen, The Netherlands	2010
Research skills Training (4.0 ECTS)	
Preparing own PhD research proposal	2007

Didactic Skills Training (26.0 ECTS)	
Lecturing in Animal Welfare, Universidad Austral de Chile	2007-2011
Lecturing in Applied Animal Welfare, Universidad Austral de Chile	2008-2011
Lecturing in Ethology and Animal Welfare, Universidad Mayor	2011
Practical assisting in Animal Welfare, Universidad Austral de Chile	2007-2011
Supervising 4 BSc and 4 MSc students	2007-2011
Coaching WSPA Concepts in Animal welfare	2009-2011
Management Skills Training (3.0 ECTS)	
Organizing Committee, Workshop for truck drivers, stock people and farmers. Temuco.	2008
Scientific Committee, meeting of Researchers on animal welfare, Valdivia.	2009
Referee for the Journal Archivos de Medicina Veterinaria	2009-2011
Education and Training	**Total 67 ECTS**

Colophon

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