Pre-slaughter cattle welfare indicators for use in commercial abattoirs with voluntary monitoring systems: A systematic review

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A B S T R A C T

Animal welfare has become an important subject of public, economic and political concern, leading to the need to validate indicators that are feasible to use at abattoirs. A systematic review was carried out, which identified 72 cattle welfare indicators (CWI) that were classified into four categories (physiological, morphometric, behavioral and meat quality). Their validity and feasibility for use in abattoirs were evaluated as potential measures of cattle welfare during transportation to the abattoir and at the abattoir itself. Several highly valid indicators were identified that are useful to assess welfare at abattoirs, including body condition score, human-animal interactions, vocalizations, falling, carcass bruising, and meat pH. In addition, some intermediate valid indicators are useful and should be investigated further. Information along the food chain could be used systematically to provide a basis for a more-risk-based meat inspection. An integrated system based on the use of key indicators defined for each inspection step with the setting of alarm thresholds could be implemented.

1. Introduction

Throughout pre-slaughter operations, animals may be challenged by stress-inducing situations, i.e., when they cannot adapt behaviorally or physiologically to environmental or physical challenges (Terlouw, 2015). Those challenges perturb homeostasis and an adaptive response is activated in an attempt to restore balance (King et al., 2006). First, physiological responses associated with emotional reactivity (heart rate and respiratory frequency) are triggered, followed by behavioral changes (Van de Water, Verjans, & Geers, 2003; Averós, Martín, Riu, Serratos, & Gosálvez, 2008; Bourguet et al., 2010; Pighin et al., 2013). Then, the activation of the hypothalamic-pituitary-adrenal axis (HPA) and the release of corticotropin-releasing hormone (CRH) activate the sympatho-adrenal component of the autonomic response increasing cortisol levels (Ferguson & Warner, 2008; Hemsworth et al., 2011; Romero, Uribe-Velásquez, Sánchez, Rayas-Amor, & Miranda-de la Lama, 2017). The adaptation process redirects energy from production to adaptation. Catecholamine release induces hypophagia and weight loss by its effects on the liver and on white and brown adipose tissue (Rabasa & Dickson, 2016). In consequence, if the stress response is

strong enough, production and meat quality will be affected (Grandin & Shivley, 2015), causing major economic losses to the industry. Overall, the poorer the welfare of the animals is, the greater the economic losses will be (Ingenbleek et al., 2013).

Significant depletion of muscle glycogen reserves pre-slaughter has a profound and well documented effect on several key meat quality attributes such as ultimate pH, tenderness, color, water-holding capacity and sensory indicators (Van de Water et al., 2003; María, Villarroel, Sañudo, Olleta, & Gebresenbet, 2003; Mounier, Dubroeuq, Andanson, & Veissier, 2006; Tadich, Gallo, Bustamante, Schwerter, & van Schaik, 2005). Additionally, Rostagno (2009) shows that stress can have a significant deleterious effect on food safety (an aspect of maximum consumer concern), providing evidence that links stress with pathogen load and shedding in farm animals, although the mechanisms underlying this effect have not been fully elucidated. Extensive research, both under experimental and commercial conditions, has shown that handling of livestock can markedly affect their stress physiology and productivity (Hemsworth et al., 2011). To understand the causes and consequences of stress at slaughter, various studies have been conducted under controlled conditions to evaluate reactions to specific

procedures (Bourguet, Deiss, Cohen Tannugi, & Terlouw, 2011). Field studies provide useful information about the effect of commercial environments and are particularly valuable where it is not possible to simulate all factors present in the environment within a controlled experiment (Jarvis, Harrington, & Cockram, 1996). Both approaches are necessary to understand how animals react to different aspects of pre-slaughter procedures (Bourguet et al., 2011). The pre-slaughter phase includes the conditions and practices between the home stall and the stunning box at the abattoir (Ferguson & Warner, 2008). During this period, animals can be exposed to a range of challenging stimuli (Ljungberg, Gebresenbet, & Aradom, 2007), making it difficult to determine which procedures contribute significantly to their stress status (Bourguet et al., 2010). Measurements of impaired biological functioning, particularly those connected to decreased health and increased physiological stress responses, can provide good corroborating evidence that welfare is compromised (Duncan, 2005). In this context, valid, feasible and easy to assess indicators are fundamental to help make it more auditable for producers and more transparent for consumers (Miranda-de la Lama et al., 2017).

Government legislation, have traditionally been the main method for ensuring or improving farm animal welfare (Bennett, 1997). Nevertheless, animal production systems which promote higher standards of animal welfare are believed to lead to higher environmental and financial costs, which will ultimately be passed onto the consumer unless subsidies or tax breaks are put in place for producers. Market-based solutions are reflected by the increase in the number of private standards introduced with many companies developing voluntary welfare monitoring systems, that ensure that producers and consumers do not have a price out of the market if any additional costs are transmitted through the supply chain (Clark, Stewart, Panzone, Kyriazakis, & Frewer, 2017). Voluntary monitoring systems, can be defined as the systematic (continuous or repeated) measurement, collection, collation, analysis, interpretation, and timely dissemination of animal-health and welfare data from defined populations (Correia-Gomes et al., 2017). For such monitoring, animal-based welfare indicators are considered the most valid, because the animals themselves are assessed (Llonch, King, Clarke, Downes, & Green, 2015). But a wide range of CWI should be considered when interpreting the responses of animals to variations in their environment, including indirect ones related to product quality. On-farm welfare assessments are the main method for assessing cattle welfare, but those assessments are labor intensive, time consuming and may even increase disease transmission within and between farms (Dalmat, Temple, Rodríguez, Llonch, & Velarde, 2009). Post-mortem meat inspection has the potential to more easily detect diseases and certain welfare conditions which may not be apparent during ante-mortem inspection of the animal upon arrival to the abattoir (Vial & Reist, 2014; Grandin, 2017). In the past, welfare audits have been developed and tested as instruments for research or commercial requirements for documenting animal welfare at the abattoir level. However, these audits are not meant to be used routinely in most veterinary surveillance systems around the world (Kristensen, Stoier, Würtz, & Hinrichsen, 2014). Therefore, there is an increased international trend to incorporate welfare indicators during meat inspection at abattoirs as a voluntary monitoring tool for cattle health (Harley, More, Boyle, O'Connell, & Hanlon, 2012; van Staaveren et al., 2017). Likewise, a relatively new idea has been proposed known as "iceberg" or "key" indicators. In their 2009 report, the Farm Animal Welfare Council (FAWC) suggested using iceberg indicators in abattoirs as a means of assessing and ensuring overall animal welfare, from the farm of origin to the abattoir (van Staaveren et al., 2017). The aim of this paper is to review current knowledge about CWI while assessing their validity (that they measure what they intend to measure) and feasibility (in terms of speed, cost and effect on normal operating procedures) of use in abattoirs. The indicators were classified into four categories (physiological, morphometric, behavioral and product quality) and qualitatively evaluated based on published literature.

2. Materials and methods

The main aim of this review is to assess indicators that scientists and other professionals currently use to measure cattle welfare and their possible application in abattoirs. Most inspections at abattoirs are focused on meat quality *peri* –or *post-mortem* in order to protect public health by ensuring food safety. Nevertheless, there is increasing interest in incorporating *pre-mortem* CWI that could indicate underlying problems (van Staaveren et al., 2017).

2.1. Search criteria and strategy

We performed a systematic review of scientific literature published between January 1995 to October 2016 based on methodology developed by Llonch et al. (2015), only including published and peer reviewed journal articles regarding the welfare assessment of cattle (calves, steers, heifers, cows and bulls). Searches were performed using the same search terms in four search engines: (1) PubMed; (2) ScienceDirect; (3) Scopus; and (4) Scielo. The search terms used (including all titles, abstracts and keywords) were: cattle OR beef cattle AND welfare OR 'animal welfare' AND transport OR lairage OR stun OR slaughter OR abattoir AND 'meat quality' AND PUBYEAR > 1994. Records from the databases were exported directly to EndNote, including articles in English or Spanish. Duplicates and documents not directly related to cattle welfare were removed.

2.2. Criteria for selection of animal-based welfare indicators

A total of 85 articles were retrieved and read to identify animal-based and product-quality based indicators associated with cattle welfare. Related indicators assessing the same welfare problem were combined to give 72 separate indicators (Tables 1 to 4). Each indicator was then allocated to a category of measurement (physiological, morphometric, behavioral and product quality). The validity and feasibility of measuring each indicator in an abattoir was categorized as high, intermediate or low, according to Llonch et al. (2015). High validity indicators were those validated in previous research through a large number of articles, which in turn had internal (e.g. sampling, measures and procedures) and external (inferences) validity, making them prone to be considered as valid indicators. Intermediate validity indicators were those that did not necessarily indicate poor welfare (e.g. body condition score). Low validity indicators were suggested but lack evidence that they actually assess welfare. Indicators with high feasibility were those that could be recorded in abattoirs, regardless of the number of animals, the space available for the animals, or the speed of the processing line. Medium feasibility indicators were those that needed special requirements (e.g. extra space or time) for appropriate assessment. Low feasibility indicators were those that could not be routinely assessed in commercial abattoirs. Where an indicator was defined as having high validity in its original setting, but did not appear likely to be valid or feasible when measured in an abattoir, we considered the possible use of intermediate validity indicators with high feasibility, or alternative novel technologies.

3. Results

The assessment of current scientific literature provided 72 indicators of cattle welfare, separated by category and feasibility in Tables 1 to 4, for a total of 22 physiological, 2 morphometric, 32 behavioral and 15 product quality indicators. Most of the studies (85%) were performed under commercial conditions, with only a few experimental ones (Apple, 1999; Apple et al., 1999; Lensink et al., 2000; María et al., 2003; King et al., 2006; Schwartzkopf-Genswein et al., 2007; Bourguet et al., 2010; Burdick et al., 2010; del Campo, Brito, Soares de Lima, Hernández, & Montossi, 2010; Alende et al., 2014; Emenheiser et al., 2014).

Table 1
Physiological indicators of cattle welfare, classified according to their validity and feasibility for use in abattoirs to assess prior cattle welfare.

Category	Subcategory	Indicator	Observation (transport T; lairage L; pre-slaughter handling PSH; slaughter and bled SB)	Commercial categories of livestock (male calves Mc; steers S; female calves Fc; heifers H; Cows; Bulls)	Validity (high H; intermediate I; low L)	Feasibility in abattoir (high H; medium M; low L)	References (first author and year of publication)
Physiological	Endocrine measures	Plasma cortisol concentration	T, L, PSH, SB	Mc, S, Fc, H, Cows, Bulls	H	L	Lensink et al., 2000; Lensink et al., 2001; Jacobsen et al., 2003; Van de Water et al., 2003; María et al., 2004; Tadicich et al., 2005; Amtmann et al., 2006; King et al., 2006; Mounier et al., 2006; Averós et al., 2008; Bourguet et al., 2010; Burdick et al., 2010; Bourguet et al., 2011; Hemsworth et al., 2011; Miranda-de la Lama, 2013; Pighin et al., 2013; Werner et al., 2013; Alende et al., 2014; Probst et al., 2014; Francisco et al., 2015; Chulayo et al., 2016; Romero et al., 2017
		Catecholamines	T, L, PSH	Mc, S, Cows	L	L	Bourguet et al., 2010; Burdick et al., 2010; O'Neill et al., 2012; Alende et al., 2014
	Fear/Excitement index and release of catecholamines	Glucose	T, L, PSH	Mc, S, Fc, H, Cows, Bulls	H	L	Jarvis et al., 1996; Van de Water et al., 2003; María et al., 2004; Tadicich et al., 2005; Amtmann et al., 2006; Averós et al., 2008; Bourguet et al., 2010; Pighin et al., 2013; Alende et al., 2014; Probst et al., 2014; Chulayo et al., 2016
	Food deprivation index	B-hydroxybutyrate	T, L	Mc, S, H, Cows	H	L	Jarvis et al., 1996; Tadicich et al., 2005; Amtmann et al., 2006; Werner et al., 2013; Van de Water et al., 2003
		Non-esterified fatty acids (NEFA)	T, L	Mc, Fc	H	L	Jarvis et al., 1996
	Indicators of fasting	Free fatty acids	L	Mc, S, H, Cows	H	L	Amtmann et al., 2006; Pighin et al., 2013; Alende et al., 2014
		Glycogen	T, L	S	H	L	Van de Water et al., 2003; María et al., 2004; Tadicich et al., 2005; Amtmann et al., 2006; Averós et al., 2008; Werner et al., 2013; Probst et al., 2014; Boles et al., 2015; Francisco et al., 2015
	Physical effort index	Creatine kinase (CK)	T, L, PSH	Mc, S, Fc	H	L	Van de Water et al., 2003; María et al., 2004; Tadicich et al., 2005; Amtmann et al., 2006; Averós et al., 2008; Werner et al., 2013; Probst et al., 2014; Boles et al., 2015; Francisco et al., 2015
		Lactate	T, L, PSH	Mc, S, Fc, H, Cows	H	L	Van de Water et al., 2003; María et al., 2004; Tadicich et al., 2005; Amtmann et al., 2006; Averós et al., 2008; Bourguet et al., 2010; Miranda-de la Lama, 2013; Alende et al., 2014; Probst et al., 2014; Boles et al., 2015; Francisco et al., 2015; Hayes et al., 2015
	Dehydration and/or hemoconcentration indicators	Lactate dehydrogenase	PSH	Mc	H	L	Francisco et al., 2015
		Packed cell volume	T, L, PSH	Mc, S, H, Cows	H	L	Jarvis et al., 1996; Tadicich et al., 2005; Amtmann et al., 2006; Pighin et al., 2013; Werner et al., 2013
	Fear/Excitement index and release of catecholamines	Total serum protein	T, L, PSH	Mc, S, H, Cows	H	L	Jarvis et al., 1996; Averós et al., 2008; Pighin et al., 2013; Werner et al., 2013; Alende et al., 2014; Francisco et al., 2015
	Dehydration and/or hemoconcentration indicators	Hematocrit	T, L, PSH	Mc, Cows, Bulls	H	L	Averós et al., 2008; Bourguet et al., 2011; Miranda-de la Lama, 2013
		Red cells	T, PSH	Mc	H	L	Averós et al., 2008; Miranda-de la Lama, 2013
		Osmolarity	L	Mc, S, H, Cows	H	L	Jarvis et al., 1996

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Table 1 (continued)

Category	Subcategory	Indicator	Observation (transport T; lairage L; pre-slaughter handling PSH; slaughter and bled SB)	Commercial categories of livestock (male calves Mc; steers S; female calves Fc; heifers H; Cows; Bulls)	Validity (high H; intermediate I; low L)	Feasibility in abattoir (high H; medium M; low L)	References (first author and year of publication)
	Immunosuppression indicators	White cells	T, L, PSH	Mc	H	L	Averós et al., 2008; Giannetto et al., 2011; Jacobsen et al., 2003; Miranda-de la Lama, 2013; Werner et al., 2013
	Immune response	IgA	PSH	Mc	H	L	Francisco et al., 2015
		Acute phase proteins (haptoglobin, serum amyloid A, fibrinogen)	T, L, PSH	Mc	I	L	Jacobsen et al., 2003; Averós et al., 2008; Giannetto et al., 2011; Werner et al., 2013; Francisco et al., 2015
	Intense physical effort index	Heart rate	T, L	Mc, Fc, Cows	L	L	Lensink et al., 2001; Van de Water et al., 2003; Schwartzkopf-Genswein et al., 2007; Bourguet et al., 2010; Giannetto et al., 2011
	Fear/Excitement markers – Emotional reactivity	Respiratory rate Rectal temperature Heat shock proteins	T, L T, L T, L, PSH	Mc Mc H, Cows, Bulls	I L I	M L L	Giannetto et al., 2011 Burdick et al., 2010; Giannetto et al., 2011 Chulayo et al., 2016

Table 2

Morphometric indicators of cattle welfare, classified according to their validity and feasibility for use in abattoirs to assess prior cattle welfare.

Category	Subcategory	Indicator	Observation (transport T; lairage L; pre-slaughter handling PSH; slaughter and bled SB)	Commercial categories of livestock (male calves Mc; steers S; female calves Fc; heifers H; Cows; Bulls)	Validity (high H; intermediate I; low L)	Feasibility in abattoir (high H; medium M; low L)	References (first author and year of publication)
Morphometric	Body condition Bodyweight	PSH	PSH	Cows, Bulls	H	H	Apple, 1999; Apple et al., 1999; Emenheiser et al., 2014
		T, PSH	T, PSH	Mc, S, H	H	M	Lensink et al., 2000; Gallo et al., 2001; Schwartzkopf-Genswein et al., 2007; Ribeiro et al., 2012; Miranda-de la Lama, 2013; Werner et al., 2013; Boles et al., 2015; Francisco et al., 2015

Table 3
Behavioral indicators of cattle welfare, classified according to their validity and feasibility for use in abattoirs to assess prior cattle welfare.

Category	Subcategory	Indicator	Observation (transport T; lairage L; pre-slaughter handling PSH; slaughter and bled SB)	Commercial categories of livestock (male calves Mc; steers S; female calves Fc; heifers H; Cows; Bulls)	Validity (high H; intermediate I; low L)	Feasibility in abattoir (high H; medium M; low L)	References (first author and year of publication)
Behavioral	Pre slaughter	Human-animal interactions	T, L, PSH, SB	Mc, S, H, Bulls, Cows	H	H	Lensing et al., 2000; María et al., 2004; Bourguet et al., 2010; Hensworth et al., 2011; Miranda-de la Lama et al., 2012; Muñoz et al., 2012; Hultgren et al., 2014; Probst et al., 2014; Hayes et al., 2015; Doyle et al., 2016; Grandin, 2017; Romero et al., 2017
		Vocalizations	T, L, PSH, SB	Mc, S, H, Bulls	H	H	Grandin, 1998; Grandin, 2001; María et al., 2004; Nanni Costa et al., 2006; Sandström, 2017; Bourguet et al., 2011; Miranda-de la Lama et al., 2012; Muñoz et al., 2012; Hultgren et al., 2014; Probst et al., 2014; Hayes et al., 2015; Romero et al., 2017
		Run	T, PSH	Mc, S, H, Bulls	H	H	Lensing et al., 2001; Sandström, 2017; Hultgren et al., 2014
		Slide	T, L, PSH, SB	Mc, S, Fc, H, Bulls, Cows	H	H	Lensing et al., 2001; Van de Water et al., 2003; María et al., 2004; Nanni Costa et al., 2006; Minka and Ayo, 2007; Sandström, 2017; Bourguet et al., 2011; Miranda-de la Lama et al., 2012; Muñoz et al., 2012; Hultgren et al., 2014; Romero et al., 2017
		Fall	T, L, PSH, SB	Mc, S, Fc, H, Bulls, Cows	H	H	Van de Water et al., 2003; María et al., 2004; Nanni Costa et al., 2006; Minka and Ayo, 2007; Sandström, 2017; Bourguet et al., 2011; Miranda-de la Lama et al., 2012; Muñoz et al., 2012; Hultgren et al., 2014; Romero et al., 2017
		Moving backwards	T, PSH	Mc, S, Fc, H, Bulls	H	H	Van de Water et al., 2003; Sandström, 2017; Bourguet et al., 2011; Muñoz et al., 2012; Hultgren et al., 2014
		Jump	T, L, PSH, SB	Mc, S, H, Bulls, Cows	H	H	Nanni Costa et al., 2006; Minka and Ayo, 2007; Sandström, 2017; Muñoz et al., 2012; Hultgren et al., 2014; Romero et al., 2017
		Kick	T, PSH	Mc, S, H, Bulls	H	H	Lensing et al., 2001; Bourguet et al., 2011; Hultgren et al., 2014
		Aggression	T, PSH	Mc, S, H, Bulls, Cows	H	H	María et al., 2004; Minka and Ayo, 2007; Miranda-de la Lama, 2013; Hultgren et al., 2014; Romero et al., 2017
		Freezing	PSH	S, H, Bulls	H	H	Hultgren et al., 2014
		Exploring	PSH	S, H, Bulls	H	H	Bourguet et al., 2011; Doyle et al., 2016
		Head movements	L, PSH	Mc, S, H, Cows	I	M	Jarvis et al., 1996; Gallo et al., 2001; Schwartzkopf-Genswein et al., 2007
		Drinking	T, L	S	H	L	Jarvis et al., 1996; Gallo et al., 2001; Lensink et al., 2001; Schwartzkopf-Genswein et al., 2007
		Rumiating	T	Mc, S, H, Cows	H	M	Schwartzkopf-Genswein et al., 2007
		Lying	T, L	Mc, S, H, Cows	H	M	Jarvis et al., 1996; Gallo et al., 2001; Lensink et al., 2001; Schwartzkopf-Genswein et al., 2007
		Standing	T, L	Mc, S, H, Cows	H	H	Schwartzkopf-Genswein et al., 2007
		Moving	T, L	Mc, S, H, Cows	H	H	Jarvis et al., 1996; Schwartzkopf-Genswein et al., 2007
		Reactivity	T, L, PSH	Mc, S, Cows	H	H	Lensing et al., 2000; King et al., 2006; Bourguet et al., 2010; Burdick et al., 2010
		Social separation	L, PSH	Cows	H	M	Bourguet et al., 2010
		Gait scoring	T, L	Mc, S, Fc, H, Cows, Bulls	I	M	Grandin, 2015; NAMI, 2016; Zimpro Corporation, 2016; Edwards-Callaway et al., 2017; Grandin, 2017
		Respiratory activity	SB	Mc, S, H, Bulls	H	H	Gallo et al., 2003; Gregory et al., 2007; Sandström, 2017; Atkinson et al., 2013; Mpanhanga and Wotton, 2015; Pérez-Linares et al., 2015; Neves et al., 2016
		Limb movement	SB	S, H, Bulls	H	H	Atkinson et al., 2013; Mpanhanga and Wotton, 2015
		Muscle tone	SB	S, H, Bulls	H	H	Atkinson et al., 2013; Mpanhanga and Wotton, 2015; Neves et al., 2016

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Table 3 (continued)

Category	Subcategory	Indicator	Observation (transport T; lairage L; pre-slaughter handling PSH; slaughter and bleed SB)	Commercial categories of livestock (male calves Mc; steers S; female calves Fc; heifers H; Cows; Bulls)	Validity (high H; intermediate I; low L)	Feasibility in abattoir (high H; medium M; low L)	References (first author and year of publication)
		Eye roll	SB	Mc, S, H, Cows, Bulls	H	H	Gallo et al., 2003; Gregory et al., 2007; Sandström, 2017; Atkinson et al., 2013; Gibson et al., 2015; Mpamhanga and Wotton, 2015
		Positive palpebral reflex	SB	S, H, Bulls	H	H	Atkinson et al., 2013; Mpamhanga and Wotton, 2015; Neves et al., 2016
		Positive corneal reflex	SB	Mc, S, H, Bulls	H	H	Gallo et al., 2003; Gregory et al., 2007; Sandström, 2017; Atkinson et al., 2013; Mpamhanga and Wotton, 2015; Pérez-Linares et al., 2015; Neves et al., 2016
		Spontaneous blinking	SB	Bulls	I	M	Sandström, 2017; Atkinson et al., 2013; Neves et al., 2016
		Time to collapse	SB	Mc, S, H, Cows, Bulls	H	H	Gregory et al., 2007; Gibson et al., 2015
		Regained posture	SB	Mc, S, H, Cows, Bulls	H	H	Gallo et al., 2003; Atkinson et al., 2013; Gibson et al., 2015; Pérez-Linares et al., 2015
	Pain responses	Responding to nostril stimulation	SB	Bulls	I	M	Neves et al., 2016
		Responding to tongue pinch	SB	Bulls	I	M	Neves et al., 2016
		General pain responses	SB	Mc, S, Fc, H, Cows, Bulls	I	M	Sandström, 2017; Atkinson et al., 2013

Table 1 shows all the physiological indicators. Of the 10 sub-categories that made up this classification, dehydration and/or hemo-concentration indicators (packed cell volume, total serum protein, hematocrit, red cells and osmolality), physical effort indices (CK, lactate, lactate dehydrogenase and acute phase proteins), and fear/excitation and emotional reactivity markers (heart rate, respiratory rate, rectal temperature and heat shock proteins), stood out in the number of indicators used to measure these subcategories (23%, 18% and 18% of the total number of indicators, respectively). However, plasma cortisol concentrations (endocrine measure), glucose (fear/excitement index and also related with the release of catecholamines) and lactate (a physical effort index) were the most used indicators among the authors corresponding to this category (83%). With respect to the morphometric category, 73% of the publications (concerning this category) used liveweight as the main morphometric indicator (Table 2).

Among behavioral indicators (Table 3), 63% corresponded to the pre-slaughter subcategory, 28% to the slaughter subcategory and only 9% to the pain responses subcategory. Human-animal interactions and vocalizations were the most common indicators during pre-slaughter (48% and 44% of authors corresponding to this category, respectively), and respiratory activity and positive corneal reflex in the slaughter subcategory (78% of authors corresponding to this category). Finally, among all the product quality indicators (Table 4), 64% were instrumental, 29% sensorial and 7% related to carcass quality. The indicators most used by the authors of this category were pH, color and bruises (68%, 47% and 34%, respectively).

4. Discussion

This review focused on identifying the main CWI used in international research, especially under commercial conditions, and assessing their validity and feasibility to promote their use at abattoirs. Systematically collected data are essential for the accurate description of health-hazard occurrence and can then contribute to the planning, implementation, and evaluation of risk-mitigation actions (Hoinville et al., 2013). Hence, animal welfare surveillance activities may provide a framework that not only enables the timely identification of hazards and threats, but also suggests approaches that either support or drive different risk management strategies to be adopted by the public and private sectors. For example, these may comprise the internalisation of risk by the private sector, stated risk mitigation and management initiatives, or complementary partnership approaches (Irvine, 2015).

4.1. Physiological indicators

Different authors have used blood parameters to assess the stress response of cattle to handling and transport (Villarreal, María, Sañudo, Olleta, and Gebresenbet, 2003b; Tadich, Gallo, Brito, & Broom, 2009). Cortisol, despite its variability and short half-life, is still one of the most used indicators, followed by packed-cell-volume (Jarvis et al., 1996; Tadich et al., 2005; Pighin et al., 2013), glucose (Van de Water et al., 2003; Averós et al., 2008; Bourguet et al., 2010; Alende et al., 2014), lactate dehydrogenase (LDH), insulin, free fatty acids (Jarvis et al., 1996), plasma activity of creatine kinase (CK), B-hydroxybutyrate (Broom, 2003; Tadich et al., 2005). More recently, haptoglobin and pigmap, a major acute phase proteins, have also been used as an indicator of poor welfare (Piñeiro et al., 2007; Averós et al., 2008; Giannetto et al., 2011; Werner et al., 2013; Francisco et al., 2015). It is a basic tenet of science (often overlooked) that measures should be adequately validated before use. This has not been done for many of the behavioral, physiological and immune measures being used (Rushen, 2003).

In some countries, governments have commissioned small periodic inspections using indicators such as cortisol, lactate and glucose (Romero et al., 2017). Nevertheless, it is not always easy to interpret cortisol levels as an indicator of the response of animals to short and long-term challenges. Adrenal cortical activity is not confined to

Table 4

Quality of the product and post-mortem indicators of cattle welfare, classified according to their validity and feasibility for use in abattoirs to assess prior cattle welfare.

Category	Subcategory	Indicator	Observation (transport T; lairage L; pre-slaughter handling PSH; slaughter and bled SB)	Commercial categories of livestock (male calves Mc; steers S; female calves Fc; heifers H; Cows; Bulls)	Validity (high H; intermediate I; low L)	Feasibility in abattoir (high H; medium M; low L)	References (first author and year of publication)
Quality of the product	Carcass quality	Bruises		Mc, S, H, Cows, Bulls	H	H	Jarvis et al., 1996; McNally and Warriss, 1996; Gallo et al., 2001; Lensink et al., 2001; Nanni Costa et al., 2006; Minka and Ayo, 2007; Sandström, 2017; Huertas et al., 2010; Strappini et al., 2010; Hoffman and Lühl, 2012; Miranda-de la Lama et al., 2012; Romero et al., 2013; Vimiso and Muchenje, 2013; Mpakama et al., 2014; Francisco et al., 2015; Cruz-Monterrosa et al., 2017
							McNally and Warriss, 1996; Lensink et al., 2000; Gallo et al., 2001; Lensink et al., 2001; María et al., 2003; María et al., 2004; Onenc and Kaya, 2004; Amtmann et al., 2006; King et al., 2006; Mounier et al., 2006; Jeleníková et al., 2008; Mach et al., 2008; Pérez-Linares et al., 2008; Bourguet et al., 2010; del Campo et al., 2010; Strappini et al., 2010; Bourguet et al., 2011; Duarte et al., 2011; Ribeiro et al., 2012; Kim et al., 2013; Miranda-de la Lama, 2013; Pérez-Linares et al., 2013; Pighin et al., 2013; Romero et al., 2013; Vimiso and Muchenje, 2013; Hou et al., 2014; Mpakama et al., 2014; Peña et al., 2014; Teke et al., 2014; Francisco et al., 2015; Chulayo et al., 2016; Romero et al., 2017
	Instrumental quality	pH		Mc, S, Fc, H, Cows, Bulls	H	H	Onenc and Kaya, 2004; Ferreira et al., 2006; King et al., 2006; Bourguet et al., 2010; del Campo et al., 2010; Bourguet et al., 2011; Chulayo et al., 2016
							Lensink et al., 2000; Gallo et al., 2001; Lensink et al., 2001; María et al., 2003; Onenc and Kaya, 2004; Ferreira et al., 2006; Panjono et al., 2009; del Campo et al., 2010; Duarte et al., 2011; Ribeiro et al., 2012; Miranda-de la Lama, 2013; Alende et al., 2014; Hou et al., 2014; Francisco et al., 2015; Chulayo et al., 2016
		Temperature		Mc, S, H, Cows, Bulls	L	L	Mpamhanga and Wotton, 2015
							Mpamhanga and Wotton, 2015
		Color		Mc, S, Fc, H, Cows	H	L	Lensink et al., 2000; Gallo et al., 2001; Lensink et al., 2001; María et al., 2003; Van de Water et al., 2003; Onenc and Kaya, 2004; Ferreira et al., 2006; Panjono et al., 2009; del Campo et al., 2010; Duarte et al., 2011; Ribeiro et al., 2012; Kim et al., 2013; Miranda-de la Lama, 2013; Pighin et al., 2013; Vimiso and Muchenje, 2013; Alende et al., 2014; Hou et al., 2014; Mpakama et al., 2014; Peña et al., 2014; Teke et al., 2014; Francisco et al., 2015; Chulayo et al., 2016
							Mpamhanga and Wotton, 2015
		Broken bones Petechial hemorrhages Water-holding capacity		S, H, Bulls S, H, Bulls	I I	M M	Lensink et al., 2001; Onenc and Kaya, 2004; del Campo et al., 2010; Duarte et al., 2011; Ribeiro et al., 2012; Kim et al., 2013; Miranda-de la Lama, 2013; Alende et al., 2014; Hou et al., 2014; Kamatara et al., 2014; Peña et al., 2014; Teke et al., 2014; Francisco et al., 2015
							Voisinet et al., 1997; Lensink et al., 2001; María et al., 2003; Onenc and Kaya, 2004; Ferreira et al., 2006; King et al., 2006; Jeleníková et al., 2008; Panjono et al., 2009; del Campo et al., 2010; Duarte et al., 2011; Ribeiro et al., 2012; Miranda-de la Lama, 2013; Alende et al., 2014; Hou et al., 2014; Kamatara et al., 2014; Peña et al., 2014; Teke et al., 2014; Francisco et al., 2015; Hayes et al., 2015; Chulayo et al., 2016
		Texture		Mc, S, H, Cows, Bulls	H	L	

(continued on next page)

Table 4 (continued)

Category	Subcategory	Indicator	Observation (transport T; lairage L; pre-slaughter handling PSH; slaughter and bled SB)	Commercial categories of livestock (male calves Mc; steers S; female calves Fc; heifers H; Cows; Bulls)	Validity (high H; intermediate I; low L)	Feasibility in abattoir (high H; medium M; low L)	References (first author and year of publication)
Post mortem	Sensorial quality	Marbling		Mc, S, H	I	M	Panjono et al., 2009; del Campo et al., 2010; Francisco et al., 2015; Hayes et al., 2015
		DFD		S, H	I	M	Voisinet et al., 1997; Kreikemeier et al., 1998; Scanga et al., 1998; Van de Water et al., 2003; Ferreira et al., 2006; Pérez-Linares et al., 2008; Pérez-Linares et al., 2013; Romero et al., 2013; Chulayo et al., 2016; Romero et al., 2017
		Tenderness		Mc, S	H	Not applicable	Lensink et al., 2001; Villarroel, María, Sañudo, García-Belenguer, et al., 2003a; Onenc and Kaya, 2004; Miranda-de la Lama, 2013; Alende et al., 2014
		Maturity		Mc, S, H	H	Not applicable	Panjono et al., 2009; Miranda-de la Lama, 2013; Hayes et al., 2015
		Odor		Mc, S	I	Not applicable	Villarroel, María, Sañudo, García-Belenguer, et al., 2003a; Onenc and Kaya, 2004; Miranda-de la Lama, 2013; Alende et al., 2014
		Flavor		Mc	H	Not applicable	Lensink et al., 2001; Villarroel, María, Sañudo, García-Belenguer, et al., 2003a; Onenc and Kaya, 2004; Miranda-de la Lama, 2013
	Rumen lesion and liver abscess scoring			Mc, S, Fc, H, Cows, Bulls	H	H	Brown et al., 1975; Rezac et al., 2014; Amachawadi and Nagaraja, 2016

adverse conditions as it occurs during courtship, mating and active food acquisition, none of which could be described as activities with adverse effects on the individual (Broom, 1988). Moreover, other factors such as sampling, movement restriction, lactation, milking, degree of habituation, hormones, infections, as well as endotoxins, can also affect cortisol levels (Sapolsky, Romero, & Munck, 2000; Trevisi & Bertoni, 2009; Blanco, Casasús, & Palacio, 2009). Normally several samples are required over time to take into account peaks and troughs and diurnal variation (Broom, 1988; Shaw & Tume, 1992). An enzyme generally utilized as an indicator of physical stress and/or muscle damage in animal production is creatine kinase (CK) (Minka & Ayo, 2009; Mpakama, Chulayo, & Muchenje, 2014; Simova, Voslarova, Vecerek, Passantino, & Bedanova, 2016). The activity of CK rises apparently as a result of an increase in the permeability of the muscle membranes induced by capture, loading and transportation (Mpakama et al., 2014). Transportation for several hours is a physical demanding factor; animals have to maintain balance and the contact between animals produces fatigue and bruising, affecting the permeability of the membranes and the liberation of the enzymes into the blood. In some experiments when the animal welfare transport order was abided with, the rise in concentration of CK in the blood was either minimal or completely absent, signifying low level of tissue damage (Hill, McManus, Brown, Playford, & Noble, 2000; Minka & Ayo, 2009). Although it is a highly used enzyme in research, it has limitations too (to be mentioned later). Each individual animal has alternative methods of coping with adversity, so using only one physiological measure of response may give the impression that most animals are not stressed (Broom, 1988; Dawkins, 1998; Galindo & Manteca, 2012). Physiological indicators are not used consistently in slaughterhouses because they are expensive, invasive, and require additional handling of the samples (Llonch et al., 2015). On the other hand, physiological indicators may not always be accurate indicators of animal welfare; the metabolism for the synthesis of some hormones (e.g. catecholamines) and the physiological status of animals per se should also be considered (O'Neill, Webb, Frylinck, & Strydom, 2012; Chulayo & Muchenje, 2015).

It is clear that all criteria used to assess welfare rely on showing some evidence of change. Change per se is not an indicator of a change in welfare since animals are continually adjusting their physiology to maintain homeostasis (Miranda-de la Lama et al., 2012). Obviously their welfare is not in a continual state of flux either, because of these continued adjustments. The important question for welfare research in both disciplines is "At what level of change is welfare at risk?" (Barnett & Hemsworth, 1990). We believe that it is important to establish clear cut-off values for each criteria for each livestock species. Furthermore, there has been a tendency in the past to rely too much on physiological, immune and behavioral measures of welfare that have not adequately been validated, and without considering health problems, which are some of the major threats to farm animal welfare (Rushen, 2003). On the other hand, extremely stress sensitive indicators may not be useful in practice, as has been the case with certain indicators such as the acute phase enzymes (e.g. Pig-Map or Haptoglobine) triggered by stressful situations due to absolutely normal management practices or by routine farm vaccination programs. The sensitivity of an indicator should be such that it is possible to reliably detect stressful situations that lead to significant suffering for animals, avoiding unnecessary alarms based on normal metabolic changes.

It seems logical that veterinarians should be heavily involved in animal welfare, but they have been historically under-represented, particularly in the English-speaking part of the world (Rushen, 2003). This has led to an underestimation of the importance of health problems as a source of animal welfare problems, and an underuse of the incidence of health problems as welfare indicators, despite early recognition of the importance of health problems for animal welfare, as reported by Broom (1986). It is clear that evaluating animal welfare is a complex task and that there is no simple and unique measure (Rushen, 2003). Consequently its evaluation must be approached from multiple

perspectives, embracing both the environment where the animal lives, the animal itself and its production. In choosing which physiological variables best measure welfare, we should not rely too heavily on “stress hormones”, as they may not be as specific to states of unpleasant motivation as we might have thought (Dawkins, 1998). As mentioned above, physiological changes can also be measured through heart and ventilation rates, as well as body temperature, which can change according to activity level and preparation for emergency action. In both situations, those variables provide information about how hard the animal is working to cope with a situation (Broom, 1991) and have often been used when analysing the effects of transport and lairage (Lensink, Fernandez, Cozzi, Florand, & Veissier, 2001; Van de Water et al., 2003; Schwartzkopf-Genswein et al., 2007; Bourguet et al., 2010; Burdick et al., 2010; Giannetto et al., 2011). At slaughter, however, measuring the heart or respiratory rate is not feasible, although the latter could be calculated indirectly based on body temperature (using a thermographic camera for example). Stressors can cause a short-lived increase in core body temperature in ruminants, which can be measured easily (Pascual-Alonso et al., 2017). There have been a number of recent studies using Infra-Red Thermography (IRT) for disease and welfare surveillance of livestock. Infra-Red Thermography involves using an infrared camera on animals in a pen, and then identifying the surface temperature of some part of the animal from the sensed wavelengths (Yazdanbakhsh, Zhou, & Dick, 2017). Using the surface of the eye is a common choice for fever surveillance; anatomically it should correlate well with the animal's core temperature, and be less affected by ambient temperatures (Okada, Takemura, & Sato, 2013). Along those lines, Burdick et al. (2010) found that ‘temperamental’ bulls may exhibit a higher temperature and respiratory response to stressors compared with ‘calm’ and ‘intermediate’ bulls.

4.2. Morphometric indicators

In beef cattle, common indicators of undernutrition are bodyweight or body condition score (BCS), although their use at slaughter level is limited since they vary in terms of animal age, sex and breed, mature size, stage of pregnancy or gut fill (Nicholson & Sayers, 1987; Morris, Kenyon, & Burnham, 2002; Coopman, De Smet, Laevens, Van Zeveren, & Duchateau, 2009; Tebug, et al., 2016; Wangchuk, Wangdi, & Mindu, 2017). Normally, at the slaughterhouse, weight loss is calculated in groups or batches, not individually. Consequently, BCS is generally used as a measure of nutritional status. Although BCS does not indicate current state of hunger, it does provide information on long-term nutritional status (Phythian, Hughes, Michalopoulou, Cripps, & Duncan, 2012). Usually the BCS estimates mobilization of energy reserves of cattle or the degree of fatness or thinness using a 5-point scale (0 = thinnest, 5 = fattest). Other scoring ranges use the 1–9 scale, in the USA, and the 0–2 scale proposed by the Welfare Quality Project (Corah, 1989; Welfare Quality, 2009).

According to Morris et al. (2002), the condition score technique is easily learned, requires no equipment and, although somewhat subjective, provides reliable results when related to subcutaneous fat cover. Apple (1999) mentioned that BCS can be useful to both cow-calf producers and slaughter-cow processors in making market decisions. Measuring BCS can be considered highly feasible, however, some scores have too many categories and suffer from inter-observer variability (Grandin, 2017). Halachmi, Klopčic, Polak, Roberts, & Bewley (2013) report that results depend on the person scoring, familiarity with the cows, and consistency between scoring periods, suggesting the use of a device for automatic (objective) monitoring of body condition, such as thermographic cameras or ultrasound scanning. However, the costs of equipment and consultants may only make it feasible for larger commercial breeding flocks, for genetic selection programs and for production systems where carcass attributes are important in classification (McGregor, 2017). In developing economies and in remote regions where new technologies are not available, low cost subjective

evaluation methods such as subjective BCS are the only alternative. Body Condition Score can also undergo sudden changes, such as during the productive cycle of the cow. That may produce a high-risk metabolic state (negative energy balance) since large amounts of body reserves are mobilized by the animal and must be metabolized, making it more prone to the onset of metabolic diseases.

4.3. Behavioral indicators

Slaughter plants are designed based on conventional architectural criteria, such as space optimization or the facilitation of human activities, but do not normally consider the behavioral needs of animals (Miranda-de la Lama et al., 2012). Complete measurements of animal welfare require observations from unloading to unconsciousness, including at the unloading bay, during lairage, moving the cattle to the stun box and at bleeding out (Hultgren, Wiberg, Berg, Cvek, & Lunner Kolstrup, 2014). Reactions to certain aspects of slaughter procedures could have consequences for subsequent slaughter stages, so improving one stage may have positive effects on subsequent stages (Bourguet et al., 2011). For cattle, loading and unloading are often more stressful than the journey itself; however, there are no regulations that define the appropriate conditions or time limits for these procedures (María, Villarroel, Chacón, & Gebresenbet, 2004). On the other hand, it should be taken into account that both the facilities available during those stages and the handling by the stockpersons will also influence animal behaviour. Several authors suggest the measurement of falls, aggression/fight, slips, jumps, baulks, reversing, mounting and vocalizations (Van de Water et al., 2003; Minka & Ayo, 2007; Bourguet et al., 2011; Hultgren et al., 2014), since they are indicators (events) associated with fear-related behavioral responses and might reflect the efficiency with which the animals are handled upon arriving at the slaughter plant (Hemsworth et al., 2011; Miranda-de la Lama et al., 2012). Loading and unloading conditions will influence the quality of lairage time; however, lairage under poor conditions will also affect animal behaviour.

Grandin (2017) mentions the wide variation among cattle behaviour during lairage, based on reports by abattoir personnel; cattle can be difficult or easy to move and the former are more likely to be abused. That may be explained by differences in emotional reactivity, i.e., the tendency to show pronounced reactions to different fear-inducing situations (Bourguet et al., 2010). The tests used to evaluate animal fear, in the human-animal relationship (HAR), can be divided into three categories: 1) evaluation of the reactions to the presence of a stationary person, 2) evaluation of the reactions to a person in movement, and 3) reactions to management (Lensink et al., 2001; Bourguet et al., 2010; Waiblinger, Menke, Korff, & Bucher, 2004). At the abattoir, these tests should normally be quite brief (Hultgren et al., 2014) and carried out on groups cattle, to eliminate stress due to social isolation, which can affect the results. This methodology has been useful to identify problems related to the design of the slaughter plants, deficiencies in selection and training of personnel responsible, staff attitude and the emotional state of the animals evaluated (Hemsworth, 2003; Hultgren et al., 2014; Romero et al., 2017).

The drive to the stunning box can be stressful, depending on the length and design of the chute and the quality of the HAR. Vocalizations due to electric prod use, balking, refusing to move, backing up and turning around are indicators that can be compared between different producers animals (Grandin, 2017). Electric goads are very stressful for cattle. According to Grandin (2010), the percentage of animals moved with an electric goad is one of five measures recorded by private industry and some governments when auditing animal welfare at slaughter plants. Previous studies have shown that increased handler interaction is correlated with an increased physiological stress response in cattle, and that tactile interactions and high-pitched or loud noises are associated with fear or stress cattle (Breuer, Hemsworth, Barnett, Matthews, & Coleman, 2000; Weeks, 2008; Hemsworth et al., 2011). Human-generated or artificial auditory interactions have also been

shown to generate increased heart rate and movement in cattle as well (Waynert, Stookey, Schwartzkopf-Genswein, Watts, & Waltz, 1999). Objective handler-behaviour observations used by several authors include tactile interactions (e.g. pushing, hitting and electric prod), auditory interactions (e.g. talking, shouting, whistling and the use of artificial noises, such as banging of pen fittings), visual interactions (e.g. waving arms or flapping objects) and contact with sensitive areas (Hemsworth et al., 2011; Hultgren et al., 2014; Doyle et al., 2016). Doyle et al. (2016) also developed a subjective assessment that evaluates the relationship between stockpersons and animals. Although this scale may be a useful way to record handler behaviour and provide a fast and effective technique for auditing, further research is needed to confirm its reliability. The quality of handling during the driving of the animals will influence the effectiveness of the stunning. Probst et al. (2014), Bourguet et al. (2011) and Romero et al. (2017) all mention that the ease with which animals are driven to the stunning box can be associated with the number of stuns required. The number of electrical prods may render subsequent stunning more difficult, or alternatively, animals that were reactive when introduced into the stunning box, could be also reactive during stunning. The stress status of the operator may play a role too (Hemsworth, 2003). Vocalizations during stunning are a useful indicator since they are related to inefficient gun calibration, lack of maintenance, deficient personnel training, presence of very excited cattle and the excessive pressure of the head brace (Grandin, 2010). As mentioned above, vocalizations in the stunning box are indicative of reactivity, illustrating that animals should be stunned without delay (Muñoz, Strappini, & Gallo, 2012). Finally, indicators such as slips, reversals, falls and jumps could also be considered when designing stunning boxes (Gallo, Teuber, Cartes, Uribe, & Grandin, 2003).

The types of behavioral observations used to assess pain can be divided into subjective or objective measures. Subjective scoring systems have been particularly popular in the veterinary literature because they are considered relatively easy to apply in a clinical setting. A well-known example for dairy cattle is gait scoring to assess lameness (Rushen, de Pasillé, von Keyserlingk, & Weary, 2008). Cattle mobility scoring is becoming one of the widely accepted method of assessing potential welfare effects. But no such tool existed two years ago for finished cattle; however, it is important to mention that several lameness studies have been conducted on farms, showing that it is a serious welfare problem (von Keyserlingk, Barrientos, Ito, Galo, & Weary, 2012; Cook, Hess, Foy, Bennett, & Brotzman, 2016). Mobility scoring is an animal-based measure. Typically it involves using a scoring system with a four point severity scale that assesses cattle mobility (gait or movement) following the pattern of movement of the limbs of cattle during locomotion (Grandin, 2015; NAMI, 2016; Zinpro Corporation, 2016). Although finished cattle can certainly suffer from lameness (with significant economic impacts for feedyards), impaired mobility has been relatively unstudied in finished cattle compared with dairy cattle (Edwards-Callaway, Calvo-Lorenzo, Scanga, & Grandin, 2017). Lameness animals that have difficulty walking can be assessed easily when they are unloaded from the trucks at the abattoir. To facilitate comparisons between slaughter plants, both producers and the meat industry should choose a common scoring tool (Grandin, 2017). Some of the challenges associated with large-scale cattle mobility scoring are consistency and subjectivity of mobility scoring, the environment in which cattle are evaluated, and the speed at which cattle must be evaluated in commercial environments (Edwards-Callaway et al., 2017). Due to this, some researchers have proposed to using the incidence of hoof problems and/or an automated monitoring system (e.g. cameras that detect lameness problems or fatigued animals –no ambulatory animals-) (Thomson, Loneragan, Henningson, Ensley, & Bawa, 2015) as a more reliable approach than gait analysis. However, there is little standardization regarding hoof lesion scoring (Rushen et al., 2008; van Staaveren et al., 2017).

Some behavioral indicators are thought to be more valid than others

(Llonch et al., 2015). While having the advantage of being non-invasive, some are more costly in terms of time required by the evaluator (María, 2017). Since the abattoir is a novel environment for cattle, as compared to the farm, behavioral CWI can only be considered to be valid at the time of inspection at the abattoir, without providing valid information about prior welfare (Visser et al., 2001). On the other hand, cattle behaviour at the abattoir could be also influenced by individual characteristics like breed (Minka & Ayo, 2007; Hoffman & Lühl, 2012; Sant'Anna & Paranhos da Costa, 2013), sex (Probst et al., 2014; Hoffman & Lühl, 2012), age (Hoffman & Lühl, 2012; Hultgren et al., 2014) and earlier experience (Lensink et al., 2000; Lensink et al., 2001; King et al., 2006; Bourguet et al., 2010; Pighin et al., 2013).

4.4. Product quality and post mortem indicators

Several studies have shown that even slight defects in beef quality indicate suffering *ante-mortem*, as corroborated by physiological, plas-matic or behavioral indicators. However, a lack of meat quality defects does not assure an absence of suffering before slaughter (María, 2008). In terms of carcass and meat quality, the most common welfare indicator associated with improving handling practices is skin lesions, such as scrapes and bruises, potential objective indicators of welfare. A bruise is defined as a visible extravasation of erythrocytes in the sub-cutis and surrounding tissue, following trauma to the body by the impact of a blunt instrument (Pilling, Vanezis, Perrett, & Johnston, 2010). The skin surface is intact but the walls of veins, venules and small arteries are torn, so blood leaks into the surrounding tissue (Barington & Jensen, 2016).

Recording carcass bruises at the abattoir may have significant potential as a resource for surveillance of welfare problems in livestock (Correia-Gomes et al., 2016). Bruises are highly valid and feasible indicators of animal welfare, indicating basic failures in the pre-slaughter logistic chain. They can help to identify the source of problems, such as electric prod usage, protruding objects or rough edges, falling, abusive stockman-ship, social mixing, or drop gates (Miranda-de la Lama et al., 2012). First it is important to establish a baseline (distinguishing between accidental and avoidable lesions), upon which improvements can be made and then to standardize assessment. To improve accuracy, the same person should do the scoring at the abattoir (Grandin, 2017). In practical terms, bruises can be separated into two categories: fresh bruises and old bruises (that occurred before lairage; Grandin, 2017). Determining the age of the bruise is important in order to associate it with pre-slaughter handling, but if transport and lairage occurs within 12 h, it is very difficult tell when a bruise was inflicted (Strappini, Metz, Gallo, & Kemp, 2009). Bruises older than 18 h are more yellow than fresh bruises (Langlois, 2007). Approximately one hour after a bruise is inflicted, changes in its gross appearance depend on the force of impact, which will affect its severity and amount of necrotic muscle tissue (Barington & Jensen, 2016). Several bovine carcass scores have been developed worldwide to be used at slaughterhouses for commercial purposes (Strappini et al., 2009). 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', 'medium' and 'heavy'. The Finnish Meat Research Institute has developed a carcass-bruising evaluation system based on the color and severity of the trauma (Honkavaara, Rintasalo, Ylonen, & Pudas, 2003). While 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, 1994; [INN] Instituto Nacional de Normalización, Chile, 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 (Strappini

et al., 2009).

Stress responses may influence post-mortem muscle metabolism and consequently, meat quality (Bourguet et al., 2010; Hemsworth et al., 2011). At the commercial level, the last pH measurement taken is one of the most important reference values to measure meat quality and is related to the depletion of glycogen reserves, and the release of lactate caused by stressful handling (Terlouw, 2015). It is also the most commonly used instrumental indicator in studies that evaluate pre-slaughter handling, because it takes into account metabolic routes and muscle energy stores. Animal transport includes the farms, intermediate points (auction markets, storage centers, classification of logistics centers, health checkpoints, logistical stopovers and resting points) and the slaughter plant (Miranda-De la Lama et al., 2014). In this context, Romero, Uribe-Velásquez, Sánchez, & Miranda-de la Lama (2013) found that animals from markets or that made stops during the journey had higher pH levels compared to those transported directly from the farm to the slaughter plant. María et al. (2003) and Ferreira et al. (2006) did not find many significant changes in meat pH in terms of journeys duration but few effects are noted when stress is mild or animals are in good health. According to Warriss, Kestin, Brown, & Wilkins (1984) glycogen resources can be restored at lairage, and cattle can recover from physical exhaustion even if they are not fed. On the other hand, Gallo, Espinoza, & Gasic (2001), Mounier et al. (2006), del Campo et al. (2010) and Teke, Akdag, Ekiz, & Ugurlu (2014) found that ultimate pH was lower when lairage was longer. Several authors suggest that the ability of cattle to rest or recover during lairage depends on the environment and the effects of feed and water restriction (Jarvis et al., 1996; Van de Water et al., 2003). Bourguet et al. (2011) report that differences in slaughter procedures and slaughter type (halal vs. conventional) can also influence ultimate pH. Overall, stressors appear to be additive and multiple stressors in the pre-slaughter period will result in a higher ultimate muscle pH than a single stressor alone (del Campo et al., 2010). Other studies have found that differences in ultimate pH are due to feeding system, temperament and breed (Lensink et al., 2000; Lensink et al., 2001; Amtmann, Gallo, van Schaik, & Tadich, 2006; Mounier et al., 2006; Ribeiro et al., 2012; Pighin et al., 2013; Mpakama et al., 2014), commercial category (Mach, Bach, Velarde, & Devant, 2008; Romero et al., 2013), and bruises (McNally & Warriss, 1996; Strappini, Frankena, Metz, Gallo, & Kemp, 2010; Vimiso & Muchenje, 2013). Hence, ultimate pH is a valid indicator to measure meat quality, being both reliable and commercially viable, to such the extent that many abattoirs systematically measure meat pH (María, 2008).

The pH of the muscle can also affect important commercial characteristics of color and water holding capacity. When the final meat pH is 5.8–6, the proteins suffer several molecular changes, including the presence of spaces (gaping) that are rapidly filled in with water (greater water retention), and are obstacles for free oxygen transport from the surface to the center of the muscle, with which the myoglobin would be transformed into metmyoglobin, giving a darker color to the meat (Terlouw, 2005). Vimiso & Muchenje (2013) found a significant negative linear effect of distance, stocking density and transportation duration on color. On the contrary, María et al. (2003) and Van de Water et al. (2003) did not find a major effect of transport on meat color. María et al. (2003) mention that no single factor is responsible for meat discoloration, which is probably due to a combination of factors associated with post-mortem changes. Other factors that have been related to an increase in both the color and the water holding capacity of the meat are the position of the animal inside the livestock vehicle (front vs back) (Van de Water et al., 2003), lairage time (Gallo et al., 2001; del Campo et al., 2010; Teke et al., 2014), stunning method (Onenc & Kaya, 2004; Kim et al., 2013), temperament of the animals (Voisinnet, Grandin, O'Connor, Tatum, & Deesing, 1997; Lensink et al., 2000; Lensink et al., 2001; King et al., 2006; del Campo et al., 2010; Ribeiro et al., 2012; Miranda-de la Lama et al., 2013; Pighin et al., 2013; Mpakama et al., 2014; Peña et al., 2014; Francisco et al., 2015),

and the carcass suspension method (Kamatara et al., 2014; Hou et al., 2014). Measurement of water holding capacity in abattoirs is limited; however, meat color could be evaluated using a color standards.

A pH above 5.8, together with a dark color and high water retention, are characteristics of DFD (dark, firm, dry) meat. That has been related to animal temperament (Voisinnet et al., 1997), commercial category (Voisinnet et al., 1997; Kreikemeier, Unruh, & Eck, 1998; Scanga, Belk, Tatum, Grandin, & Smith, 1998), poor handling during transport (Pérez-Linares et al., 2013; Romero et al., 2017), lairage (high stocking densities and climatic conditions; Kreikemeier et al., 1998; Pérez-Linares et al., 2013; Teke et al., 2014; Romero et al., 2017), time needed to enter the stunning box (Pérez-Linares et al., 2013), failed stunning (Miranda-de la Lama et al., 2012), and bruises (Romero et al., 2013; Vimiso & Muchenje, 2013). When borderline dark cutting is determined by visual means, the carcasses classified as “dark” do not necessarily exhibit true DFD characteristics. Therefore, María (2008 and 2017) suggested the possibility of detecting animals prone to this type of defective meats by infrared thermographic cameras (objective evaluation method), which is considered non-invasive. It consists in obtaining a thermal photo of the animal through an infrared camera that will give a characteristic image of candidates for DFD. This system can be installed in abattoirs and will indicate to the operators when an animal (that is going to be slaughtered) can develop abnormal meat. Therefore, it will indicate a convenient wait for its full recovery. However, both the cost of equipment and the recruitment of consultants to use the equipment (or training of staff at the abattoir) may be a limiting factor in economic terms (McGregor, 2017). Thus, a “color sampler” (subjective evaluation method) could be used instead.

The alterations of instrumental quality (pH, color, water retention and texture), also have an influence on the organoleptic properties of meat. María et al. (2003) found differences in sensory quality with respect to transport time in terms of tenderness and overall liking, while Alende et al. (2014) found the same changes in meat but related to longer lairage times. Onenc & Kaya (2004) and Hayes et al. (2015) found that both stunning method and religious slaughter affect beef tenderness. Finally, Miranda-de la Lama et al. (2013) found that scoring by taste panels was affected by the social rank of animals, specifically regarding tenderness and some odors and flavors. Although these indicators are extensively used by researchers, their feasibility of use at abattoirs is low since their evaluation methods are complex to implement commercially.

Acute and chronic acidosis, conditions that follow ingestion of excessive amounts of readily fermented carbohydrates, are prominent production problems for ruminants fed diets rich in concentrate. With acute acidosis, ruminal acidity and osmolality increase markedly as acids and glucose accumulate; these can damage the ruminal and intestinal walls, decrease blood pH, and cause dehydration, laminitis, polioencephalomalacia, and liver abscesses (Owens, Secrist, Hill, & Gill, 1998). All this leaves obvious traces in the animal's organs that can be verified during post-mortem inspection. Liver abscesses are detected only at the time of slaughter, because cattle, even those that carry hundreds of small abscesses or several large abscesses, seldom exhibit any clinical signs (Nagaraja & Chengappa, 1998). On the other hand, rumenitis lesions may serve as “timeless” pathological sequela and indicators of past chemical insults to ruminal epithelium (Rezaca et al., 2014). Although there are logistical challenges to conducting pathologic examination of the rumen at chain speed in modern packing plants (Llonch et al., 2015), there are several advantages to including rumen lesion data in conjunction with liver abscess data when monitoring ruminal health in a production system or evaluating interventions. Nevertheless, the methods and scoring systems must be well documented and consistent, and data collection must be complied over time to establish baseline or “normal” ranges (Brown et al., 1975; Rezaca et al., 2014; Amachawadi & Nagaraja, 2016).

4.5. Key indicators and future considerations

Animal welfare is an essential part of any agroecosystem. Animal production systems have always taken into account the welfare of the animals. What is new is that the concept of animal welfare has now been redefined and includes aspects (previously little considered), such as the possibility of expressing natural behaviour or the absence of negative emotional states. Therefore, current animal welfare assessment systems must take into account these new animal needs and include appropriate indicators (valid, reliable and viable) that allow to assess the physical and mental welfare of the animals. Increasing world trade associated with a growing demand for animal protein has increased the number of cattle raised, transported and slaughtered, also increasing welfare problems at various points in the supply chain (Miranda-de la Lama, 2013). As welfare is a multi-dimensional concept, it makes sense to use a multi-criteria aggregation system to assess it (Czycholl, Kniese, Schrader, & Krieter, 2017). Even though there are limitations to using CWI at slaughter plants, they could greatly improve handling by reducing stress and pain caused by poor management (Grandin, 2017). Assessment of welfare is critical to animal management, welfare surveillance, enforcement of legislation, compliance with farm assurance schemes, and welfare labelling. Along those lines, “iceberg” or “key” indicators provide an overall assessment of welfare, just as the protruding tip of an iceberg signals its submerged bulk beneath the water's surface’ (FAWC, 2009). If these indicators provide reliable information about the welfare state of animals, assessments could be limited to those specific measures, and the time taken for an assessment could be reduced accordingly (Heath, Browne, Mullan, & Main, 2014).

In this context, meat inspection is one of the most widely implemented and longest running systems of surveillance. Its primary objective is to identify animals that are not fit for human consumption and to remove their carcasses and offal from the food chain. Additional objectives are to support animal disease control and, more recently, to identify and prosecute animal welfare issues (Stärk et al., 2014). Several authors have proposed the use of a complete set of indicators to benchmark the prevalence of CWI to inform risk-based selection for inspections measuring compliance with animal welfare legislation (Støier, Larsen, Aaslyng, & Lykke, 2016). Nevertheless, both the cost and the simplicity of the assessment must be taken into account. Since meat inspection is also a resource-consuming activity, the use of key indicators has been proposed as a useful tool to provide a picture of the overall welfare of the animal and function as a warning signal for underlying problems. Provided they are shown to be valid and feasible, key indicators could substitute the current large set of measures used in welfare assessment (FAWC, 2009). An integrated system based on the use of key indicators defined for each inspection step with the setting of alarm thresholds could be implemented. This type of system enables a complete traceability of a processed batch with the record of alarm signals raised during the meat inspection process and of the management measures applied (Stärk et al., 2014).

Assessing the welfare of animals in general, while aiming for objectivity and using quantitative measures, is not entirely value free. Animal welfare is a concept that admits moral values and other more emotional preferences (Heleski & Anthony, 2012). Animal welfare scientist must comply with the moral values of society in order to generate sustainable approaches to animal welfare management (Ohl & van der Staay, 2012; Ferguson, Schreurs, Kenyon, & Jacob, 2014). Recently, instrumental and sensory meat qualities have also been correlated with ethical quality, i.e. aspects of animal welfare that may be compromised during the production process (Webster, 2001). In this context, it will be necessary to inform consumers and the meat industry that the ethical value of a product is an element of growing economic importance and an increasing business opportunity (Miranda-De la Lama, Villarreal, & María, 2014). Attention to those factors may help avoid the appearance of technical barriers to global beef trade in the future (Pighin et al.,

2013). Finally, it is worth mentioning that although animal welfare in European and English-speaking abattoirs has been evaluated and reported broadly, such data are relatively sparse in other countries. The way animals are managed and processed outside European and English-speaking markets can be markedly different in terms of scale of production, infrastructure, market requirements, the availability of manual labor and technology. As a result, research in those regions is necessary (Doyle et al., 2016; Njisane & Muchenje, 2017).

5. Conclusions

Knowledge of potential synergies and trade-offs between CWI and voluntary monitoring systems for value chain is essential for meat industry who aim to improve the level of welfare and the meat quality at pre-slaughter commercial operations. This review can serve as a tool that provides very useful indicators; however, it is also important to note that there may be variations on the effectiveness of these indicators among regions (e.g. developed vs developing world), and even in the same country (e.g. export plant vs municipal abattoirs). Even though the production of meat has become more efficient and line speeds have increased, there is still room for improvement throughout the pre-slaughter logistic chain. This systematic review identifies highly valid indicators that are useful to assess cattle welfare at abattoirs, including body condition score, human-animal interactions, vocalizations, carcass bruising and meat pH. In addition, some intermediate valid indicators (i.e., respiratory rate, body temperature, and DFD meat) are useful and should be investigated further. Data collection at commercial abattoirs can help to improve procedures. In general, information along the food chain could be used much more systematically to provide a basis for a more-risk-based meat inspection.

Conflict of interest

All authors declare that there are no present or potential conflicts of interest among the authors and other people or organizations that could inappropriately bias their work.

Acknowledgements

This study was funded by the Mexican Council for Science and Technology (CONACyT), project CB-2015-259327 for Metropolitan Autonomous University (UAM-Lerma). Many thanks to the CONACyT and National Autonomous University of Mexico (UNAM), for Scholarship PhD Program for Natiyeli Losada-Espinosa.

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