Prevalence of Potential Animal-Based Indicators of Poor Welfare Status

in

New Zealand Bobby Calves

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I hereby certify that the thesis has not been submitted for a higher degree at any University or Institution and work embodied in this thesis is my work unless noted otherwise in the acknowledgements.

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Abstract

The dairy industry is a major contributor to the New Zealand economy. Agricultural production systems have typically focussed on supply, price and competition. However, an increasing public awareness of the welfare of production animals has raised ethical concerns. Of particular concern are the calves, called bobby calves, which are surplus to the need of the dairy and beef industries and sent to slaughter at a very young age, typically four to seven days old. These calves are transported live from the farm to commercial processing plants where they are held in lairage before slaughter. Their young age at removal from the dam, mixing and transport mean that there are numerous opportunities for welfare compromise of these animals to occur.

While mortality (death and condemnation) of calves during transport and lairage is very low (e.g. 0.12% in 2016), this is the only indicator of bobby calf welfare routinely measured in New Zealand. Mortality is a crude measure of welfare and the calves that do not die before they reach the slaughter line may also experience poor welfare. The overall aim of this study was to determine the prevalence of proposed health and welfare indicators in bobby calves in lairage at commercial meat processing plant facilities in New Zealand prior to slaughter.

A systematic mapping of the literature was conducted to develop an understanding of research associated with identifying potential welfare indicators that could be used to monitor the calf welfare. The literature was systematically searched and identified a total of 99 potential nutritional, environmental, health and behavioural indicators from 253 relevant articles.

A large scale observational study conducted to assess the welfare status of bobby calves in lairage prior to slaughter at selected processing plants across New Zealand from July to October 2016. The study investigated firstly, the prevalence of potential animal-based ante-mortem indicators of calf health and welfare at the

processing plant in lairage. Secondly, selected indicators were investigated to evaluate the effect of time spent in lairage before observation and week of the study on their prevalence. The study used potential indicators identified during the systematic mapping that were able to be applied quickly and without equipment in lairage facilities of commercial meat processing plants. Observations were made at 12 meat processing plants across New Zealand, and included 102 pens of calves and 504 individual assessments that accounted for a total of 5910 calves. From this observational study, dehydration measured using a skin tent test, faecal soiling, increased respiratory rate and ocular/nasal discharge were found to be prevalent in bobby calves in lairage and may be useful indicators of aspects of calf welfare. The logistic regression model recognised the prevalence of nasal discharge, faecal soiling and dehydration (two second cut off) increased later in the season. The prevalence of increased respiration rate was inversely proportional to time in lairage, and the prevalence of lying down increased with time in lairage. Future research is recommended to identify the aetiology of indicators of health and behaviour in order to reduce the number of calves experiencing compromised welfare.

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List of Abbreviations

>	Greater than
<	Less than
≤	Less than or equal to
NZ	New Zealand
MPI	Ministry for Primary Industries
95% CI	95% Confidence interval
OR	Odds ratio
SE	Standard error

Chapter 1: General Introduction

1.1 Bobby calf industry in New Zealand

The dairy industry is a major contributor to the New Zealand economy contributing $7.8 billion, or 3.5%, to New Zealand's total gross domestic product (Ballingall & Pambudi, 2017). Agricultural production systems have previously concentrated on supply, price and competition; however, increasing public awareness of, and attention to, the welfare of production animals have fuelled the ethical concerns which are increasingly requiring food to be produced with consideration of the welfare of the animal (Blokhuis *et al.*, 2003; Main *et al*., 2003; Duncan, 2005; Sejian *et al.*, 2011).

Bobby calves (*Bos taurus*) are calves surplus to the needs of the dairy industry that are sent for slaughter, typically, between four to seven days of age (Thomas & Jordaan, 2013) and include: male calves, freemartins and the unrequired heifer calves. In recent years, around 2 million bobby calves have been slaughtered annually in New Zealand (NZ) (Cook, 2014). Concern about the slaughter of these young calves and their treatment is a persistent issue for the NZ dairy industry (Renaud et al., 2017). While public awareness about the welfare of bobby calves in NZ is not novel (e.g. Wesselink, 1998), recent media attention surrounding the mistreatment of these animals has refuelled concerns (e.g. "Calves 'beaten to death'", 2015; "Shocking video of dairy industry", 2015; Piddock, 2016).

Dairy production in NZ is strongly seasonal producing large numbers of calves during the main calving period (July to September). In 2014 only 26% of the 4.1 million calves born in NZ were required as replacement dairy heifers (Hickson et al., 2015). The excess of calves cannot be absorbed into the dairy or beef market due to low replacement rates in the dairy herd (20-25%; MPI, 2017) and a limit on land area for rearing beef cattle. The remaining calves are either sold to be raised as beef cattle, or sold as bobby calves and sent for slaughter (Wesselink, 1998; Stafford et al., 2001; Mellor, 2011). Furthermore, the jersey breed has been deemed unsuitable to be reared as beef cattle as consumers do not like the appearance of the meat due to its distinctive yellow coloured fat, as well as slower growth rates and lighter carcasses (Burke et al., 1998). Hence, by the very nature of the NZ dairy industry there will always be unwanted calves and this problem will not be removed through the use of sexed sorted semen so that only female calves produced and rearing excess calves for beef (McDermott et al., 2005; Cook, 2014; Jolly, 2016).

The majority of these unwanted calves are transported live to the meat processing plant for commercial slaughter and products are processed into: veal for human consumption, leather goods, pet food and byproducts for the pharmaceutical industry (Jayathilakan *et al.,* 2012; Alao *et al.,* 2017). Calves sent for slaughter are at risk of compromised welfare due to the young age at which they are transported, held in lairage and slaughtered (Fisher *et al.,* 2009). Throughout the supply chain, whether it is on farm, during transportation or at the meat processing plant, there are numerous opportunities for welfare compromise to occur. However, the only routinely collected data relating to bobby calf welfare in NZ is mortality rate (MPI, 2017), and more recently, the presumed cause of death, determined via post-mortem, for calves that died or were condemned, i.e. humanely killed before slaughter due to poor state (MPI, 2017).

It is important to note that the mortality rate in the NZ bobby calf population is very low and has decreased over recent years. For example, only 0.12%, or 2,255 of the 1.94 million calves presented for processing in 2016 were condemned or were found dead upon arrival at the slaughterhouse or during their time in lairage (MPI, 2017). This is an improvement upon 2015 when the mortality rate was 0.25%, or 5,390 of the 2.17 million calves slaughtered (MPI, 2017). The marked improvements in mortality rate are likely due to: improved education throughout the supply chain of those responsible for handling and caring for young calves, the introduction of new regulations and increasing awareness surrounding welfare of bobby calves (MPI, 2016).

The use of mortality as a standalone indicator of calf welfare across the supply chain is insufficient. While mortality is likely to be an objective indicator of animal welfare in this context, it is the least desired outcome as it means welfare compromise was not recognised and/or appropriate steps were not taken to intervene before death. This indicates the potential for welfare compromise of significant intensity and/or duration in both the animals that died/were condemned and possibly other animals that went undetected. Thus there is a need to scientifically assess calf welfare to increase our understanding of welfare compromise and improve calf welfare.

1.2 Current Understanding of Animal Welfare

Animal welfare is a multidimensional concept incorporating ethical, cultural, religious and political concerns, scientific knowledge, economic considerations, historical factors and legal requirements (Sutherland, 1999; Main *et al.,* 2003; Main, 2010; OIE, 2012). Improving animal welfare encompasses identifying issues that may influence the animal's welfare and promoting corrective measures that will benefit the animal (Barnett & Hemsworth, 2009). Thus, assessment of welfare status using scientific principles and methods is important if animal welfare is to improve. There are currently three major scientific orientations to understanding animal welfare which focus on 1) biological function, 2) affective state, or 3) natural state/living (Fraser *et al.*, 1997).

The biological functioning orientation considers welfare to be good when the animal is healthy, able to grow, reproduce, and have good productivity, as well as being hindered by as few physiological and behavioural disruptions as possible (Fraser, 2003). The concept of biological functioning can help to understand the animal's welfare state through physiological and behavioural responses and thus associates poor welfare with biological dysfunction (Broom & Johnson, 1993; Hemsworth & Coleman, 1998). Aligned to this orientation, Duncan (2005) proposes a hierarchy of biological needs that affect the animal's welfare. These can be ranked as life-sustaining, health-sustaining, and comfort-sustaining needs (Hurnik & Lehman, 1988; Duncan, 2005). For example, water is characterized as a life-sustaining need; without water, the animal's welfare is markedly reduced and if deprivation is sustained, the animal will die (Duncan, 2005). Maintenance of welfare is less dependent on provision of health-sustaining needs and even less so on meeting comfort needs. However, these health and comfort needs drive the physiological or behavioural responses of the animal ensuring their requirements are met and safeguarding the animal's welfare.

The second orientation, affective state, puts emphasis on the animal's mental experiences or/and emotions, in particular stressing the potential for an animal to have both negative and positive experiences (Mellor *et al.*, 2009). Negative experiences are likely experienced when the primary affects experienced are unpleasant (Hemsworth *et al.,* 2015). Such experiences can include pain, weakness, breathlessness, thirst, hunger and fear (Fraser, 2003; Mellor & Beausoleil, 2015) – see Figure 1.1 for a more detailed list of possible affective experiences. Under the affective state view the emphasis should be on achieving good welfare by reducing negative emotions and by providing opportunities for positive encounters with humans, other animals and the environment (Mellor *et al.,* 2009). The affective state orientation describes an animal with good welfare as one which experiences positive emotions involving social and environmental interactions and minimal negative experiences (Mellor *et al.*, 2009).

Increasingly, the affective state orientation is integrated with biological functioning due to affective experiences often being considered a result of biological functioning (Hemsworth *et al.,* 2015). Improvements in animal welfare are possible when the dynamic interactions between biological function and affective state are considered (Boissy *et al.,* 2007; Barnett & Hemsworth, 2009; Green & Mellor, 2011; Hemsworth *et al.,* 2015). Integration of such orientations is supported by the understanding that inputs from the surrounding environment determine the animal's internal physical/functional state (Hemsworth *et al.,* 2015). The animal's perception of its external environment affects the physical/functional state within the animal and produces affective experiences (Mellor, 2012, Hemsworth *et al*., 2015; Mellor, 2016). For example, during prolonged water deprivation the affective experience of thirst is generated when animals are dehydrated (physical state) and osmoreceptors in the hypothalamus transmit signals which results in the conscious awareness of thirst (Mellor & Beausoleil, 2015). Negative effects such as thirst motivate survival-focused behaviours and can result in positive experiences including wetting/quenching pleasures of drinking water, driven by the thirst (Mellor & Beausoleil, 2015). Thus, indicators of adequate/good biological function have the potential to be used to cautiously and scientifically infer the likely associated affective experiences to assess an animal's welfare status (Mellor, 2016).

The third orientation of 'natural living' reflects the principle that animals should be able to express normal behaviours and live in the most natural state possible (Fraser, 2003). Furthermore, animals should be provided with opportunities to express natural behaviours beneficial to their welfare, rather than abnormal or detrimental behaviours, e.g. pain behaviours (Fraser, 2003). Hence, welfare compromise is relative to how the conditions in which the animal is kept deviate from the "wild" state of the species and the degree to which the animal is or is not able to demonstrate its natural behaviours (Mellor, *et al.*, 2009). For example, calves reared non-intensively and raised on their dam will feed at multiple times per day as a reaction to thirst and hunger (Fraser, 2008). Intensively kept calves are often restricted in when they are fed and the method of feeding. Providing such calves with ad libitum food and artificial teats (rather than a bucket) has been demonstrated to initiate the increased release of digestive hormones and has been associated with weight gain (Fraser, 2008). While the concept of natural living highlights potential benefits associated with the provision of opportunities to engage in natural behaviours (Mellor, 2015), this orientation has proven less useful than the biological functioning and affective state orientations when seeking defensible scientific assessment of animal welfare (Hemsworth *et al.,* 2015).

The orientation underpinning the welfare assessment undertaken in this thesis (Five Domains model; see Section 1.3) reflects the integration of the biological functioning and affective state orientations to animal welfare.

1.3 Scientific Assessment of Animal Welfare

According to the integrated biological functioning/affective state approach adopted here, animal welfare is a state within an animal which relates to the integrated affective outcome of the animal's subjective experiences at the particular time (Mellor *et al*., 2009; Mellor, 2016; Beausoleil & Mellor, 2017). As the mental experiences that most directly influence welfare status are subjective and internal, it is impossible to measure animal welfare state. Instead, the welfare of an animal is assessed through the use of observed indicators of welfare which are known to reflect a physiological state and the likely mental experience (Beausoleil & Mellor, 2017). Thus, scientific welfare assessment often involves using observable and measurable indicators considered to reflect different mental experiences (Wemelsfelder & Mullan, 2014; de Graaf *et al.,* 2017).

A variety of behavioural and physiological measures can be used to assess the welfare of animals (Mellor et al., 2009). Such assessments previously focussed on input-based measures such as features of the physical environment, resources provided to the animals and management inputs (Wemelsfelder & Mullan, 2014; Waran & Randle, 2017). While such inputs are often more reliably assessed and easier to measure, they should actually be considered as risk factors which have the ability to influence an animal's welfare rather than direct reflections of welfare status (AWIN, 2015).

Animal-based indicators, also considered as outputs, reflect an animal's response to its surrounding environment and can be evaluated through the use of "qualitative or quantitative physical, physiological, pathophysiological, biochemical, immunological, neurological and behavioural indicators" (Beausoleil & Mellor, 2017, p 4). While animal-based indicators allow for the most direct assessment of the animal's experiences, relationships between many animal-based indicators and resource-based or management-based indicators have been scientifically established, facilitating the use of the latter as proxies of the former (Beausoleil & Mellor, 2017). Both animal and resource based indicators have been developed for other species such as sheep and dairy goats (Battini *et* al., 2014; Llonch *et al.,* 2015), and a number of potential indicators of welfare have been described for cattle (e.g. Winckler *et al.,* 2003; Knierim & Winckler, 2009; Miranda-de la Lama *et al.,* 2012). However, little consideration has been given to their use in the context of assessing bobby calf welfare.

Welfare assessment must be based on credible scientific premises where physical/function and behavioural indices reflect their associated affective states (Mellor, 2017). Such is the basis of the Five Domains model, as originally described by Mellor & Reid (1994) and more recently enhanced by Mellor & Beausoleil (2015) (Figure 1.1). This model facilitates the identification of both positive and negative impacts affecting an animal's welfare status (Mellor & Beausoleil, 2015; Mellor, 2017). The model comprises four physical or functional domains, 'nutrition', 'environment', 'health', and 'behaviour', and a fifth domain which alludes to the animal's mental state inferred from observation of indicators of the animal's physical/functional state (Mellor & Beausoleil, 2015). The Five Domains model provides a systematic means to practically assess welfare using a variety of observable parameters (Mollenhorst *et al.,* 2005). In the context of this thesis, welfare indicators have been organized according to the Five Domains Model to ensure systematic assessment of calf welfare.

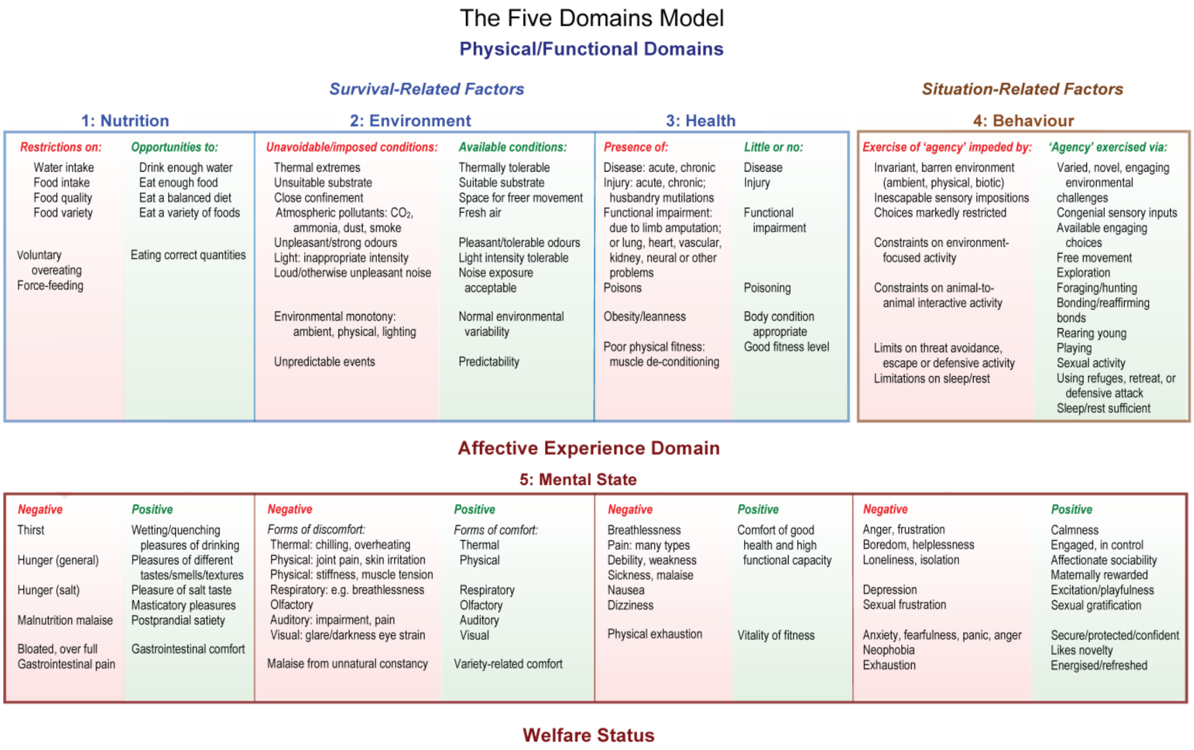


Figure 1.1: The most current version of the Five Domains model as described in Mellor & Beausoleil 2015 (sourced from: Mellor, 2017).

1.4 New Zealand legislation, regulations and codes relevant to bobby calf welfare

The Animal Welfare Act (1999, amended 2015) aims to safeguard the welfare of bobby calves by establishing rudimentary obligations of the persons in charge of the animals; since 2015, the act also allows for the development of specific regulations that are directly enforceable. New regulations relating to calves, introduced in 2016 and 2017, cover fitness for, and duration of, calf transport; prohibition of killing calves by blunt force trauma to the head, except in emergency situations; maximum time off feed before slaughter; appropriate facilities for loading and unloading calves onto and off transportation; and protection from adverse weather in the form of shelter (MPI, 2016). In addition, the current Codes of Welfare that apply to dairy cattle and calves, issued by Ministry for Primary Industry (MPI) National Animal Welfare Advisory Committee (NAWAC), expand the obligations under the Animal Welfare Act to provide minimum standards of care (NAWAC, 2016). While these are not legally enforceable, they can be used to indicate a breach of the Animal Welfare Act.

It is important to note that at the time the research described in this thesis was done, the bobby calf industry was, and still is, in an environment of change because of the incremental introduction of these new regulations. A consequence of this is that it is difficult to compare data collected prior to 2016 with those collected in 2016 and beyond.

1.5 Study Objectives

This study was conducted in order to gain an understanding of the current welfare status of bobby calves in the lead up to slaughter. The overall aim of this study was to determine the prevalence of proposed health and welfare indicators in bobby calves in lairage at commercial meat processing plant facilities in New Zealand prior to slaughter. More specifically, the research objectives were to:

- Systematically map the literature to gain an understanding of the national and internationally published research in order to determine potential animal- and resource-based indicators for assessing bobby calf welfare.
- Assess bobby calves in lairage to determine the prevalence of potential animal-based welfare indicators.
- Examine the effects of time held in lairage before being observed and the week of study on prevalent calf welfare indicators.

The findings from the study will be used to establish a set of potential welfare indicators so that evidence-based recommendations can be proposed for assessment of bobby calf welfare in lairage, with the ultimate objective of improving bobby calf welfare in NZ.

1.6 Thesis Outline

This thesis consists of four chapters. The introductory chapter introduces the aims and research objectives of this study. Chapter two is a systematic mapping of the existing literature to collate information about potential welfare indicators relating to bobby calves. Chapter three presents the results of the cross-sectional study completed in meat processing plants across NZ where data were collected to determine the prevalence of a variety of potential welfare indicators for bobby calves in lairage. Finally, chapter four offers concluding remarks, implications of the results with recommendations and offers suggested potential areas of further investigation. Chapters two and three are intended to be prepared as manuscripts for publication. A list of the relevant references cited in each chapter can be found at the end of the thesis.

Chapter 2: Systematic Mapping of Published Research on Potential Indicators of Welfare in Bobby Calves

2.1 Objective

To develop an understanding of the existing global research associated with identifying potential welfare indicators of calf welfare that could be used in a practical sense to monitor the calf welfare.

2.2 Introduction

Most of New Zealand's dairy industry runs on seasonal milking which means that large numbers of calves are born each year between July and September. Approximately a quarter of the calves will be kept as replacements (Hickson *et al.,* 2015). Female calves that are not retained as replacements and males are sold into the beef industry or sold as 'bobby calves' to be slaughtered for human consumption, pet food and/or animal products (Wesselink, 1998). Bobby calves in New Zealand can be legally sold at four days of age and are commonly transported for slaughter at this age, presenting particular risks to their welfare (Wesselink, 1998).

Improving animal welfare encompasses identifying issues that may influence the animal's welfare and promoting corrective measures that will benefit the animal (Barnett & Hemsworth, 2009). Concerns for the welfare of calves in the dairy industry, in particular bobby calves, have driven the interest in developing specific welfare assessment schemes. An array of health, behavioural and environmental parameters can potentially be used as indicators of animal welfare in such schemes (Mollenhorst *et al.*, 2005). Application of these kinds of parameters may increase our understanding of the requirements of the particular species and group of animals, facilitate routine assessment of animal welfare in industry and support the development of policy and legislation to safeguard animal welfare (Edwards, 2007).

Parameters potentially indicative of animal welfare can be divided into resourced-based indicators and animal-based indicators. Resource-based indicators describe features of the environmental surroundings and the management systems.

Although resource-based indicators are not a direct assessment of an animal's welfare, they are factors relating to the environment which are considered to affect the welfare of an animal (Mollenhorst *et al.,* 2005; AWIN, 2015). These indirect indicators relate to an animal's welfare by reflecting the suitability of inputs, including resources and management provision (Pritchard *et al*., 2005). Another way to think about resource-based indicators is in terms of informing the possible risks that may compromise or enhance an animal's welfare, rather than actually assessing the animal's welfare state (Rousing, et al., 2001; Pritchard, *et al*., 2005). In contrast, animal-based welfare indicators reflect more direct observations of an animal's reaction to a specific environment or management system (Mollenhorst, *et al.*, 2005; Pritchard, *et al*., 2005).

Concerns around the welfare of bobby calves is not new and given the growing interest in identifying welfare compromise and enhancement in a practical sense (Edwards, 2007), as well as the broad scope of the research associated with animal welfare published to date, there is a need to recognise publications associated with this topic to identify the areas that have been previously analysed and understood as well as the areas where there are knowledge gaps. There is increasing work being done to identify indicators of negative and positive welfare states in production animals (e.g. Llonch, *et al*., 2015). However, there has not been a systematic mapping of the published literature on this subject. The use of systematic mapping provides a structured method to gather the research evidence required to outline and appraise the current literature surrounding calf welfare. The methodical systems used reduce bias when identifying and evaluating studies to be included in the review by following documented protocol that allow for repeatability (Sargent & O'Conner, 2014). Systematic mapping of the literature provides a defined library of the current information on which decisions can be made, as well as identifying areas where research could be conducted to improve knowledge (O'Connor & Sargeant, 2014).

This chapter presents a systematic mapping review of animal-based and ante-mortem indicators of welfare that could be used to assess the welfare of bobby calves in lairage at commercial abattoirs in New Zealand. The overall aim was to develop an understanding of the existing global research associated with identifying potential welfare indicators of calf welfare that could be used in a practical sense to monitor the calf welfare.

2.3 Materials and Methods

The question driving the systematic mapping was:

"What is the current state of published research related to neonatal animal welfare?"

To address the question a four step process was followed (see Figure 2.1). Step one was to systematically search the literature through selected databases. The second step was to screen the title and abstract from all the articles returned in the search for inclusion or exclusion. Step three was a full article screen where relevance was considered. The final step was to manually search the references of the returned articles and retrieve any deemed relevant to bobby calf welfare.

2.3.1 *Search Procedure*

A search was conducted with the use of electronic databases available through Massey University. The search was carried out in January 2017 and allowed for the retrieval of experimental and observational studies of calf and/or cow (*Bos taurus*) welfare, including: journal articles, conference proceedings, book chapters, thesis dissertations, literature reviews, and government reports. Four electronic databases (Discover, Web of Science, Scopus and Google Scholar) were searched over all available years.

The defined search terms were derived with assistance of a librarian with knowledge in scientific research to limit the number of irrelevant articles returned, as well as to maximise the accuracy of return. The search terms were:

> *calf OR calves OR bovine OR 'bos taurus' AND bobby OR young OR neonatal AND assess* OR indicator* OR evaluat* AND 'animal welfare' OR 'calf welfare' OR welfare OR treatment OR mistreatment OR cruelty AND transport* OR abattoir* OR slaughter* OR lairage*

The article retrieval process allowed for all article types and all study designs to be selected without restrictions on year of publication or language limitations. The articles returned from the searches of the individual databases were saved as separate Endnote X7 libraries to enable the articles to be assessed.

2.3.2 *Eligibility Criteria & Selection of Relevant Articles*

The scope of this review included quantitative research which investigated welfare indicators. Relevant articles were selected using the following inclusion criteria: a publication in English; and academically published as peer reviewed journal articles, conference proceedings, book chapters, thesis dissertations, systematic literature reviews or government reports. While all study types were considered, primarily, experimental and observational studies of calf welfare outlining either animal-based or resource-based welfare indicators were retrieved. Returned articles outlining welfare indicators for any type of production animal were considered. A pre-screening of the literature, not completed systematically but rather to grasp an idea on the current work, illustrated that there is very limited research on welfare indicators in bobby calves. For this reason, articles returned during the literature searches that outlined welfare indicators in other production animals were included. Similarly, to avoid excluding any potentially relevant indicators that have not yet been address in young calves, animals of different ages, not just neonates, were considered.

The first stage of the review process involved assessing articles for relevant titles or abstracts, and the irrelevant documents or duplicates were removed at this stage.

Articles were deemed irrelevant if they were not looking at aspects of welfare that bobby calves may experience, for example pain behaviours due to husbandry procedures in lambs or the use of growth hormones in veal calves to induce growth. Articles that did not directly address potential indicators of welfare were deemed irrelevant at this point. Secondly, the references from all relevant articles were checked through a manual search to identify any articles that may be of use and were missed by the electronic search. The final screening of articles involved all relevant articles being assessed and the applicable information extracted and assembled into a simple database (Microsoft Excel). The final screening excluded articles that were not primary searches, i.e. narrative literature reviews, book chapters and government reports. The reference lists from the articles remaining after the primary screenings were re-analysed and relevant articles were included in the database.

2.3.3 *Data Extraction and Evaluation*

Relevant studies were examined and information was collected in order to identify animal-based and resource-based welfare indicators of calf welfare. The data extracted from the relevant articles included: authors, location of data collection (country and situation), year in which the study was conducted, year the article was published, the design of the study, the sample size and sampling methodology, and the indicator(s) of welfare on which the study focused.

The relevant indicators sourced from the returned literature were then categorized according to the four physical domains of welfare ('Nutrition', 'Environment', 'Health', 'Behaviour') as described in The Five Domains of Welfare Compromise; this model was devised by Mellor & Reid (1994) and later extended by Mellor & Beausoleil (2015). These four domains focus on physical and/or functional disruptions to an animal's nutritional and hydration status (Domain 1), physical and sensory environment (Domain 2) and health/function (Domain 3), as well as the animal's behavioural interactions with the environment and other animals (Domain

4). The fifth domain, 'mental state' reflects the affective experiences which are consequences of four physical/functional domains and as such was not a category. Information about physical/functional changes, reflected by changes in the indicators observed, can then be used to cautiously draw conclusions about the animal's mental experiences and thus its welfare state (Mellor and Beausoleil, 2015).

2.4 Results

2.4.1 *Descriptive characteristics of published literature regarding bobby calf welfare*

Initial searches from all four electronic databases retrieved a total of 4,864 articles. Figure 2.1 describes the search and retrieval process used to obtain relevant articles. After reviewing the titles and abstracts of all the returned articles, the number of relevant articles was reduced to 301 articles, or 6.2% of 4,864 articles initially retrieved. A further 75 were removed because they were duplicates (i.e. found in one or more database) leaving 226 original articles, or 4.6% of the 4,864 initially retrieved. When inclusion/exclusion criteria were applied to the 226 articles, 100 were removed because they were narrative review articles (n=59), the full text could not be retrieved (n=23), were irrelevant after subsequent consideration (n=15), or not primary publications (n=3). At the end of the process there were 126 articles describing welfare indicators, or 2.6% of those initially retrieved. Of the relevant articles, 13% (n=16) were retrieved by a single search engine, 51% (n=64) were retrieved by two search engines, and 36% (n=45) by all four search engines. A summary of the total number of articles returned, and the number of relevant articles sourced from each search engine is presented in Table 2.1.

At the secondary screening, which involved the articles being re-read and assessed for relevance, 14 articles were removed. The remaining 112 articles were then assessed and the references examined to determine any potentially relevant articles that may not have been returned in the initial database searches. Manual searching of the reference list recognised a further 361 potential articles addressing aspects of bobby calf welfare. The removal of articles that were already sourced in the initial search (n=47), irrelevant articles (n=74), narrative review articles (n=53) and those where the full article was unable to be sourced (n=46), resulted in 141 relevant publications. Thus, the final review consisted of 253 articles that were considered to contain relevant information regarding welfare indicators in young calves.

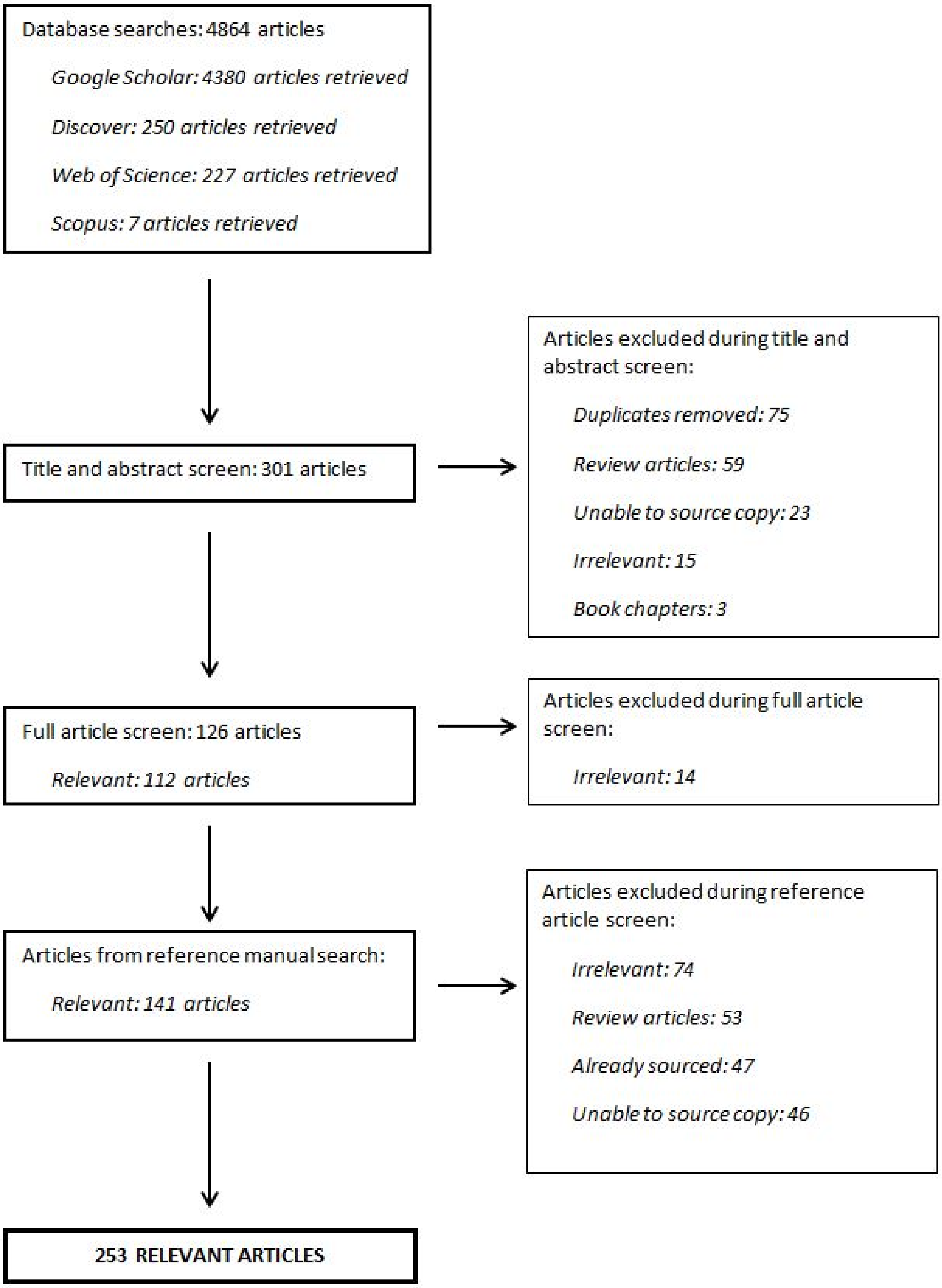


Figure 2.1: Outline of the systematic review process

Table 2.1: Total number of publications and number of relevant articles retrieved from each database

Database	Articles retrieved	Relevant Articles	Percentage accuracy
Web of Science	227	68	30
Scopus	7	4	57
Discover	250	83	33
Google Scholar	4380	102	2.3

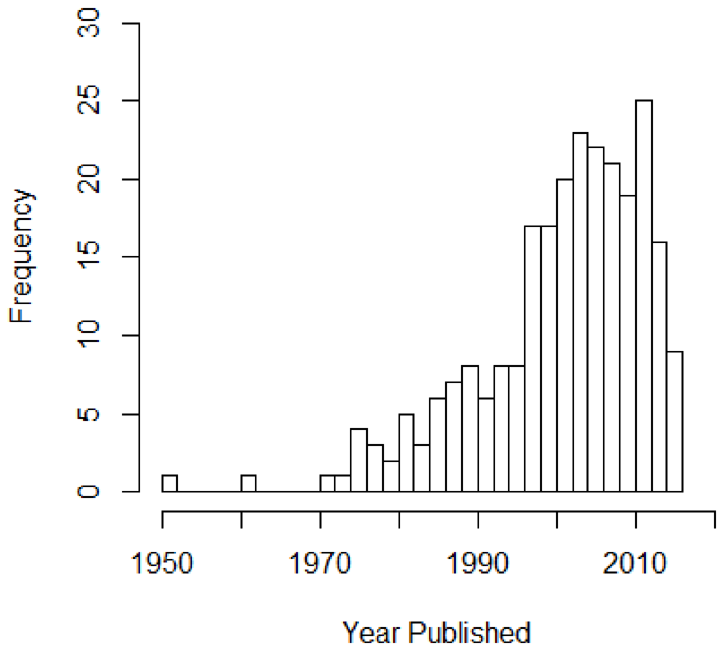
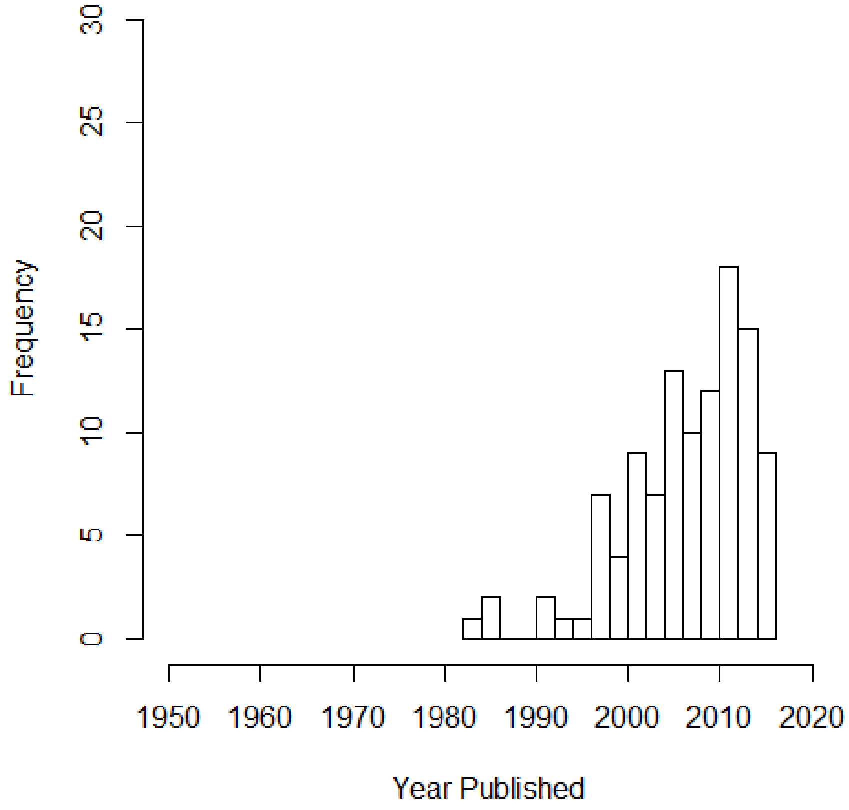


Figure 2.2: Number of relative articles by year of publication



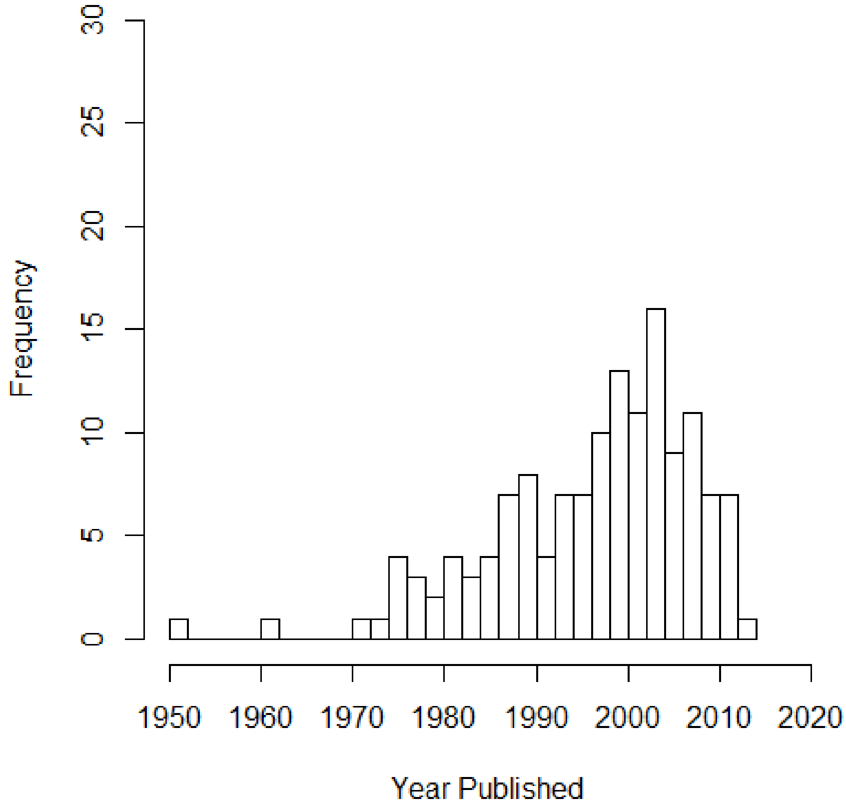


Figure 2.3: Frequency of relevant articles by year of publication for the articles retrieved in the initial search of the literature (histogram on left) and articles retrieved from references of the original articles (histogram on right).

The year in which relevant articles were published ranged from 1951 through to 2016. Of the relevant articles, more than half of these (53%) have been published during or after 2003 (Figure 2.2). Figure 2.3 illustrates the difference in year of publication between articles retrieved in the initial search and articles retrieved during searches of the references. The median year of publication of original articles is 2008 compared to the median year of 1999 from the articles retrieved during the manual search of the references of the original articles. Table 2.2 describes the classification of the studies, where, the majority of relevant articles were prospective and conducted in cows (*Bos taurus*), with a range of population age and sample size.

2.4.2 *Welfare indicators*

A total of 99 different welfare indicators were identified from the 253 articles retrieved for this review (Tables 2.3 to 2.6). A total of 121 different studies included indicators of nutritional/hydration status and identified 4 different means of assessing welfare, including body weight (n=75), feeding (n=27) and water supply (n=9). Sixteen resource-based indicators and one animal-based indicator (slipping) of welfare relating to the physical or sensory environment were identified in the articles retrieved. Resource based indicators included space allowance (n=28) and ambient temperature (n=39). Thirty-two indicators of health/functional status were identified, including physiological parameters such as blood components (n=149), body temperature (n=48), heart rate (n=38), dehydration (n=19), diarrhoea (n=19), and nasal discharge (n=13). Finally, a total of 47 behavioural indicators reflecting animals' interactions with the environment, other animals and humans were identified from the articles; these included oral behaviours (n=33), lying (n=55) and vocalising (n=25).

Table 2.2: Number and percentage of studies classifed by key features of study design. Data from 253 studies returned from a systematic review of literature.

Category	Number of studies (%) (n=253)
Study design	
Cross-sectional: measurements made at a single point in time	52 (20.6)
Prospective: measurements taken as time progressed during the study	195 (77.1)
Retrospective: exposure and outcome determined before study	6 (2.4)
Country study conducted	
Unknown	68 (26.9)
USA	21 (8.3)
New Zealand	18 (7.1)
Canada	17 (6.7)
Australia	14 (5.5)
France	14 (5.5)
Italy	11 (4.3)
England	10 (4.0)
The Netherlands	9 (3.6)
Ireland	7 (2.8)
Spain	7 (2.8)
Denmark	6 (2.4)
Netherlands, France & Italy	6 (2.4)
Europe	5 (2.0)
Sweden	5 (2.0)
Germany	4 (1.6)
Japan	4 (1.6)
Switzerland	4 (1.6)
Austria	3 (1.2)
Chile	3 (1.2)
Scotland	3 (1.2)
Finland	2 (0.8)
Bangladesh	1 (0.4)
Belgium	1 (0.4)
Czech Republic	1 (0.4)
Netherlands	1 (0.4)
New Zealand & Canada	1 (0.4)
Norway	1 (0.4)
Poland to Italy	1 (0.4)
Serbia	1 (0.4)

Category	Number of studies (%) (n=253)
Slovakia	1 (0.4)
Turkey	1 (0.4)
USA & Wales	1 (0.4)
USA, Canada & Australia	1 (0.4)
Animal species study conducted on	
Cows	217 (85.8)
Sheep	14 (5.5)
Pigs	9 (3.6)
Cows & Sheep	4 (1.6)
Buffalo	2 (0.8)
Cows & Pigs	2 (0.8)
Horses	2 (0.8)
Cows & Buffalo	1 (0.4)
Cows, Sheep & Goats	1 (0.4)
Quail	1 (0.4)
Age of Animal	
Unknown	38 (15.0)
≤3 weeks	66 (26.0)
3 weeks - 6 months	45 (17.8)
6 months - 1 year	25 (9.9)
>1 year	44 (17.4)
Numerous ages	35 (13.8)
Sample number	
Unknown	6 (2.4)
Individual animals:	
0 – 20	59 (23.3)
21 – 50	63 (24.9)
51 – 100	45 (17.8)
101 – 200	29 (11.5)
200 – 500	13 (5.1)
501 – 1000	5 (2.0)
1001 – 5000	6 (2.3)
5000+	5 (2.0)
Consignment of animals:	
0-100	9 (3.6)
100-200	7 (2.8)
200+	6 (2.3)

Table 2.3: Animal- and Resource-based indicators of Nutritional or Hydration status addressed in articles retrieved during the systematic mapping (n=253)

Welfare Indicator	Number of Studies (%) n=253	Reference
Body Weight	75 (29.7)	Blaxter & Wood, 1951; Kirton & Paterson, 1973; Stephens & Toner, 1975; Webster, et al., 1978; Crookshank *et al.,* 1979; Kertz *et al.,* 1984; de Wilt, 1985; Kent & Ewbank, 1986b; Veissier *et al.,* 1989; Schrama, 1992; Lidfors, 1993; Scott & Christopherson, 1993; Stull & McDonough, 1994; Rushen & de Passillé, 1995; Fernandez *et al.,* 1996; Schrama *et al.,* 1996; Knowles *et al.,* 1997; Mogensen *et al.,* 1997; Molony & Kent, 1997; Constable, et al., 1998; Todd, 1998; Veissier *et al.,* 1998; Wesselink, 1998; Grasso *et al.,* 1999; Jago *et al.,* 1999; Knowles *et al.,* 1999; Munksgaard *et al.,* 1999; Ekpe & Christopherson, 2000; Lensink *et al.,* 2000a; Morisse *et al.,* 2000; Todd *et al.,* 2000; Fregonesi & Leaver, 2001; Grigor *et al.,* 2001; Stafford *et al.,* 2001; Veissier *et al.,* 2001; Diesch, 2002; Gottardo *et al.,* 2002; Ibáñez *et al.,* 2002; Xiccato *et al.,* 2002; Arthington *et al*., 2003; Hänninen *et al.,* 2003; Parker *et al.,* 2003; Panivivat *et al.,* 2004; Gupta *et al.,* 2005; Hänninen *et al.,* 2005; Smulders *et al.,* 2006; Tapkı *et al.,* 2006; Gånheim *et al.,* 2007; Gupta *et al.,* 2007; Schwartzkopf-Genswein *et al.*, 2007; Buckham Sporer *et al.*, 2008; Jasper *et al.,* 2008; Pritchard *et al.,* 2008; Webster *et al.,* 2008; Petherick *et al.,* 2009; White *et al.,* 2009; de la Fuente *et al.,* 2010; Earley & Murray, 2010; Cafazzo *et al*., 2012; Duve *et al.,* 2012; Earley *et al.,* 2012; Gonzalez, et al., 2012a; Gonzalez, et al., 2012b; Marques *et al.,* 2012; Prevedello *et al.*, 2012; Stockman *et al.,* 2012; Webb *et al.,* 2012; Fisher *et al.,* 2014; Goldhawk *et al.*, 2014; Bernardini *et al*., 2015; Llonch *et al.,* 2015; Lürzel *et al,*. 2015a
Feeding	27 (10.7)	Kirton & Paterson, 1973; Olsen *et al.,* 1980; Kertz *et al.,* 1984; Veissier *et al.,* 1989; Kooijman *et al.*, 1991; Schrama, 1992; Schrama *et al.*, 1993a; Schrama *et al.,* 1993b; Scott & Christopherson, 1993; Rushen & de Passillé, 1995; Schrama *et al.*, 1995; Molony & Kent, 1997; Ekpe & Christopherson, 2000; Morisse *et al.,* 2000; Todd *et al.,* 2000; Boissy *et al.,* 2001; Fregonesi & Leaver, 2001; Margerison *et al.,* 2003; Parker *et*

Welfare Indicator	Number of Studies (%) n=253	Reference
		al., 2003; Howard, 2004; Brown-Brandl *et al.,* 2005; Sandström, 2009; Krachun *et al.,* 2010; Bähler *et al.,* 2012; Ahsan *et al.,* 2014; Bergman *et al.,* 2014; Stanković *et al.,* 2014
Body Condition Score	10 (4.0)	Fregonesi & Leaver, 2001; Diesch, 2002; Huxley *et al.,* 2004; Regula, et al., 2004; Pritchard, et al., 2008; Petherick *et al.,* 2009; Bršččić *et al.,* 2012; Takáčová *et al.,* 2012; Bergman *et al.,* 2014; Llonch *et al.*, 2015
Water supply	9 (3.6)	Kertz *et al.,* 1984; Schrama *et al*., 1993b; Parker *et al*., 2003; Howard, 2004; Parker *et al*., 2004; Garner, 2005; Sandström, 2009; Bähler *et al*., 2012; Ahsan *et al*., 2014

Table 2.4: Animal- and Resourced-based indicators of Environment-related physical/functional status addressed in articles retrieved during the systematic mapping (n=253)

Welfare Indicator	Number of Studies (%) n=253	Reference
Ambient Temperature	39 (15.4)	Ames & Insley, 1975; Webster *et al.,* 1978; Vermorel *et al.,* 1983; Webster *et al.,* 1985; Kent & Ewbank, 1986a; Vermorel *et al*, 1989; Schrama, 1992; Schrama *et al.*, 1993a; Schrama *et al.,* 1993b; Scott & Christopherson 1993; Stull & McDonough, 1994; Schrama *et al.*, 1995; Knowles *et al.*, 1997; Jacobson & Cook, 1998; Knowles *et al.*, 1999; Diesch, 2002; Ibáñez *et al.,* 2002; Mohr *et al.,* 2002; Zähner *et al.,* 2004; Brown-Brandl *et al.,* 2005; Earley, et al., 2006; Earley & O'Riordan, 2006; Averos *et al.*, 2007; Fazio *et al.,* 2008; Webster *et al.,* 2008; Earley & Murray, 2010; Stewart *et al.,* 2010; Stockman *et al.,* 2011; Uetake *et al.,* 2011; Cafazzo *et al.,* 2012; Earley *et al.,* 2012; González *et al.,* 2012a; González *et al.,* 2012b; Weschenfelder *et al.*, 2012; Stockman *et al.,* 2013; Goldhawk *et al.*, 2014; Bernardini *et al.,*2015; Wickham *et al.,* 2015
Space allowance	28 (11.1)	Kent & Ewbank, 1986a; Tarrant *et al.*, 1988; Lidfors, 1993; Fisher *et al.*, 1997; Jensen *et al.*, 1997; Mogensen *et al.,* 1997; Todd, 1998; Grasso *et al.,* 1999; Jensen, 1999; Jensen & Kyhn, 2000; Bokkers & Koene, 2001; Ibáñez *et al.,* 2002; Grigor *et al.,* 2004; Garner, 2005; Tapkı, et al., 2006; Gupta *et al.,* 2007; Sandström, 2009; White et al., 2009 Brščić *et al.,* 2011; Uetake, et al., 2011; Bähler *et al.*, 2012; Brščić *et al.*, 2012; Gonzalez *et al.,* 2012b; Leruste *et al.*, 2012b; Abdelfattah *et al.,* 2013; Bergman *et al.,* 2014; Leruste *et al.,* 2014; Cózar *et al.,* 2016
Relative Humidity	22 (8.7)	Webster *et al.,* 1985; Schrama *et al.*, 1993a; Schrama *et al.,* 1993b; Stull & McDonough, 1994; Knowles *et al.,* 1999; Zähner *et al.*, 2004; Brown-Brandl *et al.,* 2005; Earley *et al.,* 2006; Earley & O'Riordan, 2006; Averos *et al.*, 2007; Fazio *et al.*, 2008; Webster *et al.,* 2008; Earley & Murray, 2010; Stewart *et al.*, 2010; Stockman *et al.,* 2011; Uetake *et al.,* 2011; Cafazzo *et al.*, 2012; Weschenfelder *et al.*, 2012; Stockman *et al.*, 2013; Goldhawk *et al.*, 2014; Bernardini *et al.,*2015; Wickham *et al.*,

Welfare Indicator	Number of Studies (%) n=253	Reference
		2015
Environmental Conditions	12 (4.7)	Scott & Christopherson, 1993; Regula *et al.,* 2004; Brown-Brandl *et al.,* 2005; Garner, 2005; Earley *et al.,* 2006; Earley & O'Riordan, 2006; Pettiford *et al.,* 2008; Earley & Murray, 2010; Cafazzo *et al.,* 2012; Earley *et al.*, 2012; Leruste *et al.*, 2012b; Leruste *et al.,* 2014
Flooring	12 (4.7)	Stull & McDonough, 1994; Fisher *et al.*, 1997; Mogensen *et al.*, 1997; Haley *et al.*, 2001; Hänninen *et al.*, 2003; Garner, 2005; Sandström, 2009; Brščić *et al.*, 2011; Brščić *et al.*, 2012; Stockman *et al.,* 2013; Ahsan *et al.*, 2014; Leruste *et al.*, 2014
Bedding	7 (2.8)	Kent & Ewbank, 1986a; Mogensen *et al*., 1997; Howard, 2004; Panivivat *et al*., 2004; Regula *et al.*, 2004; Sandström, 2009; Peli *et al.*, 2016
Wind Speed	6 (2.4)	Ames & Insley, 1975; Webster *et al.*, 1978; Webster *et al.*, 1985; Schrama *et al.,* 1993b; Brown-Brandl *et al.,* 2005; Webster *et al.,* 2008
Housing	4 (1.6)	Tarrant *et al*., 1988; Dalmau *et al.,* 2009; Stanković *et al*., 2014; Peli *et al.*, 2016
Light levels	2 (0.8)	Stull & McDonough, 1994; Garner, 2005
Noise	2 (0.8)	Todd, 1998; Sandström, 2009
Noxious Gases	2 (0.8)	Stull & McDonough, 1994; Panivivat *et al.*, 2004
Wind chill	2 (0.8)	Ames & Insley, 1975; Webster *et al.*, 2008
Cleaning	1 (0.4)	Bokkers & Koene, 2001
Shade	1 (0.4)	Brown-Brandl *et al.*, 2005
Slipping	1 (0.4)	Ahsan *et al.,* 2014

Welfare Indicator	Number of Studies (%) n=253	Reference
Rainfall	1 (0.4)	Webster *et al*., 2008
Vibration	1 (0.4)	Van De Water *et al*., 2003a

Table 2.5: Animal- and Resource-based indicators of Health/Functional status addressed in articles retrieved during the systematic mapping (n=253)

Welfare Indicator	Number of Studies (%) n=253	Reference
Blood components	149 (58.9)	Willet & Erb, 1972; Stephens & Toner, 1975; Hudson *et al.,* 1976; Johnston & Buckland, 1976; Cabello *et al.,* 1977; Crookshank *et al.,* 1979; Olsen *et al.*, 1980; Kelley *et al.,* 1981; Olsen *et al.*, 1981; Braun *et al.*, 1982; McVeigh *et al.*, 1982; Mormede *et al.*, 1982; Vermorel *et al.*, 1983; Blecha *et al.,* 1984; de Wilt, 1985; Cole *et al.,* 1986; Fell & Shutt, 1986; Kent & Ewbank, 1986a; Kent & Ewbank, 1986b; Kenny & Tarrant, 1987; Murata *et al.,* 1987; Fröhli & Blum, 1988; Rulofson *et al.*, 1988; Tarrant *et al.,* 1988; Veissier & le Neindre, 1988; Becker *et al.*, 1989; Locatelli *et al.*, 1989; Murata, 1989; Vermorel *et al.*, 1989; Groutides & Michell, 1990; Murata & Hirose, 1990; Murata & Hirose, 1991; Lay *et al.*, 1992; Tarrant *et al.*, 1992; Scott & Christopherson, 1993; Murata & Miyamoto, 1993; Stull & McDonough, 1994; Warris *et al.*, 1994; Cooper *et al.,* 1995; Jarvis *et al.,* 1996; Schrama *et al.,* 1996; Amadori *et al.,* 1997; Fisher *et al.,* 1997; Knowles *et al.*, 1997; Mackenzie *et al.,* 1997; Molony & Kent, 1997; Veissier *et al.,* 1997; Constable *et al*., 1998; Schrader & Todt, 1998; Todd, 1998; Veissier *et al*., 1998; Wesselink, 1998; Cockram *et al.,* 1999; Grasso *et al.*, 1999; Knowles *et al.*, 1999; Munksgaard *et al.*, 1999; Ekpe & Christopherson, 2000; Lensink *et al.,* 2000a; Morisse *et al.*, 2000; Palme *et al.*, 2000; Todd *et al.,* 2000; Grigor *et al.,* 2001; Jacor *et al.,* 2001; Lensink, et al., 2001a; Lensink, et al., 2001b; Veissier *et al.*, 2001; Diesch, 2002; Stafford *et al.,* 2001; Gottardo *et al.*, 2002; Ibáñez *et al.*, 2002; Mattiello *et al.,* 2002; Mellor *et al.,* 2002; Arthington *et al.,* 2003; Parker *et al*., 2003; Van de Water *et al.*, 2003a; Van de Water *et al.,* 2003b; Diesch *et al.,* 2004; Grigor *et al.,* 2004; Maria *et al.,* 2004; Odore *et al.,* 2004; Panivivat *et al*., 2004; Parker *et al.*, 2004; Zähner *et al.,* 2004; Garner, 2005; Gupta *et al.,* 2005; Hänninen *et al*., 2005; Napolitano *et al.,* 2005; Pregel *et al.,* 2005; Raussi, 2005; Stanger *et al.*, 2005; Tadich *et al.*, 2005; van Reenen *et al*., 2005; Earley *et al.*, 2006; Earley & O'Riordan, 2006; Smulders *et al.,* 2006; Wernicki *et al.,* 2006; Averos *et al*., 2007; Buckham Sporer *et al.,* 2007; Gånheim *et al.,* 2007; Gupta *et al.*, 2007; Schaefer *et al.,* 2007;

Welfare Indicator	Number of Studies (%) n=253	Reference
		Schwartzkopf-Genswein *et al.,* 2007; Stewart *et al.,* 2007; Svensson *et al.,* 2007; Buckham Sporer *et al.,* 2008; Candiani *et al.,* 2008; Fazio *et al.,* 2008; Pettiford *et al.,* 2008; Pritchard *et al.*, 2008; Riondato *et al.*, 2008; Webster *et al.,* 2008; Bokkers *et al.*, 2009; Petherick *et al.*, 2009; Tadich *et al.*, 2009; Uetake *et al.*, 2009; Bourguet *et al.*, 2010; De la Fuente *et al.,* 2010; Earley & Murray, 2010; Ishiwata & Kariya, 2010; Miranda-de la Lama *et al.,* 2010; Stewart *et al.*, 2010; Giannetto *et al.*, 2011; Hemsworth *et al.,* 2011; Stockman *et al.,* 2011; Uetake *et al.*, 2011; Cafazzo *et al.*, 2012; Earley *et al.*, 2012; Marques *et al.*, 2012; Miranda-de la Lama *et al.,* 2012; Prevedello *et al.*, 2012; Schaefer *et al.*, 2012; Stockman *et al.*, 2012; Weschenfelder *et al.*, 2012; Abdelfattah *et al.*, 2013; Barrier *et al.,* 2013; Stockman *et al.*, 2013; Goldhawk *et al.*, 2014; Cucuzza *et al.,* 2014; Fisher *et al.,* 2014; Jongman & Butler, 2014; Bernardini *et al.,* 2015; Lürzel *et al.,* 2015a; Lürzel *et al.,* 2015b; Wickham *et al.,* 2015; Cózar *et al.*, 2016
Body temperature	48 (19.0)	Willet & Erb, 1972; Webster *et al.,* 1978; Crookshank *et al.,* 1979; Olsen *et al.,* 1981; McVeigh *et al.*, 1982; Vermorel *et al.,* 1983; Kenny & Tarrant, 1987; Vermorel *et al.*, 1989; Schrama, 1992; Schrama *et al.,* 1993a; Stull & McDonough, 1994; Jacobson & Cook 1998; Todd, 1998; Wesselink, 1998; Knowles *et al.,* 1999; Ekpe & Christopherson, 2000; Todd *et al.,* 2000; Grigor *et al.*, 2001; Stafford *et al.,* 2001; Diesch, 2002; Van de Water *et al.,* 2003a; Diesch *et al.,* 2004; Grigor *et al.,* 2004; Zähner *et al.*, 2004; Brown-Brandl *et al.*, 2005; Earley *et al.,* 2006; Earley & O'Riordan, 2006; Gupta, et al., 2007; Schaefer *et al.,* 2007; Stewart *et al.,* 2007; Svensson *et al.,* 2007; Buckham Sporer *et al.,* 2008; Pettiford *et al.,* 2008; Pritchard *et al.*, 2008; Webster *et al.,* 2008; Uetake *et al.,* 2009; Earley & Murray, 2010; Giannetto *et al.,* 2011; Stockman *et al.,* 2011; Cafazzo *et al.,* 2012; Earley *et al.*, 2012; Schaefer *et al.,* 2012; Barrier *et al.,* 2013; Stockman *et al.,* 2013; Ahsan *et al.*, 2014; Fisher *et al.,* 2014; Bernardini *et al.,* 2015; Cramer & Stanton, 2015; Wickham *et al.,* 2015

Welfare Indicator	Number of Studies (%) n=253	Reference
Heart Rate	38 (15.0)	Blaxter & Wood, 1951; Stephens & Toner, 1975; McVeigh *et al.,* 1982; Kenny & Tarrant, 1987; Lay *et al.*, 1992; Hopster & Blokhuis, 1994; de Passillé *et al.*, 1995; Jensen *et al.,* 1997; Knowles *et al.,* 1997; Constable *et al.,* 1998; Jacobson & Cook 1998; Wesselink, 1998; Cockram *et al.*, 1999; Grigor *et al.,* 2001; Lensink *et al.,* 2001a; Lensink *et al.,* 2001b; Mohr *et al.,* 2002; Van de Water *et al.,* 2003a; Van de Water *et al.,* 2003b; Grigor *et al.,* 2004; Waiblinger *et al.*, 2004; Zähner, et al., 2004; Raussi, 2005; van Reenen *et al.,* 2005; Færevik *et al.,* 2006; Lauber *et al.,* 2006; Schwartzkopf-Genswein *et al.,* 2007; Pritchard *et al.*, 2008; Uetake *et al.*, 2009; Stewart *et al.,* 2010; Giannetto *et al.*, 2011; Stockman *et al.,* 2011; Stockman *et al.,* 2013; Clapp *et al.,* 2015; Lürzel *et al.,* 2015a; Lürzel, et al., 2015b; Wickham *et al.*, 2015
Post Mortem	34 (13.4)	Kirton & Paterson, 1973; McCausland *et al.*, 1977; Wiepkema *et al.*, 1987; Tarrant *et al.*, 1988; Becker *et al.,* 1989; Tarrant *et al.*, 1992; Jarvis *et al.,* 1995; Fernandez *et al.*, 1996; Jarvis *et al.,* 1996; Molony & Kent, 1997; Grandin, 1998a; Veissier *et al.,* 1998; Lensink *et al.*, 2000a; Morisse *et al.*, 2000; Bokkers & Koene, 2001; Lensink *et al.,* 2001b; Stafford *et al.,* 2001; Veissier *et al.,* 2001;Gottardo *et al.*, 2002; Mattiello, et al., 2002; Xiccato *et al.,* 2002; Van de Water *et al.*, 2003b; Grigor *et al.,* 2004; Maria *et al.*, 2004; Dalmau *et al.*, 2009; Sandström, 2009; De la Fuente *et al.,* 2010; Miranda de la Larna *et al.,* 2010; González *et al.*, 2012b; Leruste *et al.*, 2012a; Prevedello *et al.,* 2012; Stockman *et al.*, 2012; Weschenfelder *et al.,* 2012; Ahsan *et al.,* 2014; Llonch *et al.,* 2015
Dehydration	19 (7.5)	Jarvis *et al.,* 1996; Knowles *et al.,* 1997; Constable *et al.*, 1998; Wesselink, 1998; Knowles *et al.,* 1999; Grigor *et al*., 2001; Stafford, et al., 2001; Diesch, 2002; Xiccato *et al*., 2002; Moore *et al.*, 2003; Parker *et al.,* 2003; Parker *et al.,* 2004; Fazio *et al.*, 2005; Tadich *et al.*, 2005; Schaefer *et al.*, 2007;Pritchard *et al.*, 2008; Tadich *et al.,* 2009; Schaefer *et al*., 2012; Jongman & Butler, 2014
Diarrhoea	19 (7.5)	Hudson *et al*., 1976; Kertz *et al*., 1984; Webster *et al*., 1985; Groutides & Michell, 1990; Stull & McDonough, 1994; Todd, 1998; Morisse *et al*., 2000; Mohr *et al*., 2002; Gånheim *et al.*, 2007; Schaefer *et al.*, 2007; Svensson *et al*., 2007; Uetake *et al*., 2011;

Welfare Indicator	Number of Studies (%) n=253	Reference
		Prevedello *et al.*, 2012; Schaefer *et al.*, 2012; Abdelfattah *et al.*, 2013; Thomas & Jordaan, 2013; Ahsan *et al.*, 2014; Cramer & Stanton, 2015; Llonch *et al.,* 2015
Lameness	14 (5.5)	Winckler & Willen, 2001; Fregonesi & Leaver, 2001; Mülleder *et al.*, 2003; Huxley *et al.*, 2004; Regula *et al.*, 2004; Napolitano *et al.*, 2005; Dalmau *et al.*, 2009; Ahsan *et al.*, 2014; Bergman *et al.*, 2014; Bršćić *et al.*, 2011; Bršćić *et al.*, 2012; González *et al.*, 2012b; Llonch *et al.*, 2015; Takáčova *et al.*, 2012
Coughing	13 (5.1)	de Wilt, 1985; Lidfors, 1993; Smulders *et al.*, 2006; Schaefer *et al.*, 2007; Svensson *et al.*, 2007; Bršćić *et al.*, 2012; Leruste *et al.*, 2012a; Prevedello *et al.*, 2012; Schaefer *et al.*, 2012; Takáčová *et al.*, 2012; Abdelfattah *et al.*, 2013; Cramer & Stanton, 2015; Llonch *et al.*, 2015
Nasal Discharge	13 (5.1)	de Wilt, 1985; Schaefer *et al.*, 2007; Svensson *et al.*, 2007; Bršćić *et al.,* 2011; Bršćić *et al.*, 2012; Leruste *et al.,* 2012a; Prevedello *et al.*, 2012; Schaefer *et al.*, 2012; Takáčová *et al.*, 2012 Abdelfattah *et al.,* 2013; Ahsan *et al.*, 2014; Cramer & Stanton, 2015; Llonch *et al.,* 2015
Injury	12 (4.7)	Huxley *et al.,* 2004; Regula *et al.*, 2004; Smulders *et al.*, 2006; Candiani *et al.*, 2008; Dalmau *et al.*, 2009; Bršćić *et al.*, 2011; Uetake *et al.,* 2011; Takáčová *et al.,* 2012; Ahsan *et al.*, 2014; Bergman *et al.*, 2014; Stanković *et al.*, 2014; Llonch *et al.*, 2015
Mortality	12 (4.7)	Webster *et al.,* 1985; Stull & McDonough, 1994; Molony & Kent, 1997; Morisse *et al.*, 2000; Cave *et al.,* 2005; Večerek *et al.,* 2006; Ortiz-Pelaez *et al.*, 2008; Dalmau *et al.,* 2009; White *et al.*, 2009; González *et al.*, 2012b; Thomas & Jordaan, 2013; Peli *et al.*, 2016
Cleanliness	11 (4.3)	Webster *et al.*, 1985; Fregonesi & Leaver, 2001; Gottardo *et al.*, 2002; Huxley *et al.*, 2004; Panivivat *et al.*, 2004; Regula *et al.,* 2004; Napolitano *et al.*, 2005; Smulders *et al.*, 2006; Takáčová *et al.*, 2012; Llonch *et al.,* 2015; Peli *et al.*, 2016
Respiration Rate	9 (3.6)	Blaxter & Wood, 1951; Willet & Erb, 1972; Crookshank *et al.*, 1979; Brown-Brandl *et*

Welfare Indicator	Number of Studies (%) n=253	Reference
		al., 2005; Pritchard *et al*., 2008; Dalmau *et al*., 2009; Giannetto *et al.*, 2011; Brščić *et al*., 2012; Leruste *et al.*, 2012a
Disease	7 (2.8)	Webster *et al.*, 1985; Wesselink, 1998; Schaefer *et al*., 2007; Svensson *et al*., 2007; Schaefer *et al.*, 2012; Thomas & Jordaan, 2013; Stanković *et al.*, 2014
Ocular Discharge	7 (2.8)	de Wilt, 1985; Prevedello *et al.,* 2012; Takáčová *et al.*, 2012; Abdelfattah *et al*., 2013; Ahsan *et al*., 2014; Cramer & Stanton, 2015; Llonch *et al.,* 2015
Demeanour	6 (5.4)	Schaefer *et al.*, 2007; Svensson *et al.,* 2007; Prevedello *et al.*, 2012; Schaefer *et al.*, 2012; Takáčová *et al*., 2012; Llonch *et al.,* 2015
Eyes	6 (5.4)	Constable *et al*., 1998; Stafford *et al*., 2001; Stewart *et al.*, 2007; Stewart *et al.,* 2008; Stewart *et al.*, 2010; Takáčová *et al.*, 2012
Hampered breathing	6 (5.4)	de Wilt, 1985; Schaefer *et al.,* 2007; Svensson *et al.*, 2007; Brščić *et al.*, 2012; Prevedello *et al*., 2012; Schaefer *et al.*, 2012
Infrared Thermography	5 (2.0)	Stafford *et al*., 2001; Schaefer *et al.*, 2007; Stewart *et al*., 2007; Stewart *et al*., 2008; Schaefer *et al.*, 2012
Shivering	5 (2.0)	González-Jimenez & Blaxter, 1962; Vermorel *et al*., 1983; Vermorel *et al*., 1989; Dalmau *et al.*, 2009; Llonch *et al.*, 2015
Number of teeth	4 (3.6)	Todd, 1998; Wesselink, 1998; Todd *et al*., 2000; Stafford *et al.,* 2001
Faecal soiling	3 (1.2)	Kertz *et al.*, 1984; Moore *et al.*, 2003; Panivivat *et al.,* 2004
Morbidity	3 (1.2)	Molony & Kent, 1997; Schwartzkopf-Genswein *et al.*, 2007; White *et al.*, 2009
Skin irritation	3 (1.2)	Brščić *et al.*, 2012; Takáčová *et al.*, 2012 ; Llonch *et al.*, 2015
Umbilical cord	3 (1.2)	Todd, 1998; Stafford *et al.*, 2001; Cramer & Stanton, 2015
Bursitis	2 (0.8)	Brščić *et al.*, 2011; Brščić *et al.*, 2012

Welfare Indicator	Number of Studies (%) n=253	Reference
Ear score	2 (0.8)	Abdelfattah *et al*., 2013; Cramer & Stanton, 2015
Panting	2 (0.8)	Dalmau *et al.,* 2009; Llonch *et al.*, 2015
Vulvar Discharge	2 (0.8)	Ahsan *et al.*, 2014; Llonch *et al.*, 2015
Endoparasitism	1 (0.4)	Llonch *et al*., 2015
Electroencephalogram	1 (0.9)	Hänninen *et al*., 2005

Table 2.6: Animal-based indicators of behavioural interaction with the environment, other animals or humans addressed in articles retrieved during the systematic mapping (n=253)

Welfare Indicator	Number of Studies (%) n=253	Reference
Lying	55 (21.7)	de Wilt, 1985; Kent & Ewbank, 1986a; Kent & Ewbank, 1986b; Veissier *et al*., 1989; Vermorel *et al.*, 1989; Mellor *et al.,* 1991; Tarrant *et al*., 1992; Schrama *et al.,* 1993a; Stull & McDonough, 1994; Schrama *et al.*, 1995; Jarvis *et al*., 1996; Fisher *et al.*, 1997; Mogensen *et al.,* 1997; Molony & Kent, 1997; Todd, 1998; Cockram *et al.,* 1999; Grasso *et al.*, 1999; Knowles *et al.*, 1999; Munksgaard *et al*., 1999; Haley *et al.*, 2000; Bokkers & Koene, 2001; Fregonesi & Leaver, 2001; Grigor *et al.,* 2001; Haley *et al*., 2001; Lensink *et al*., 2001b; Veissier *et al.*, 2001; Ibáñez *et al*., 2002; Mattiello *et al*., 2002; Mohr *et al*., 2002; Margerison *et al.*, 2003; Mülleder *et al.*, 2003; Van de Water *et al.*, 2003a; Grigor *et al.*, 2004; Herskin *et al*., 2004; Panivivat *et al.*, 2004; Regula *et al.*, 2004; Zähner *et al.*, 2004; Raussi, 2005; Garner, 2005; Hänninen *et al.,* 2005; Tapkı *et al*., 2006; Schwartzkopf-Genswein *et al*., 2007; Candiani *et al.,* 2008; Webster *et al.*, 2008; Bokkers *et al*., 2009; Westerath *et al*., 2009; Ishiwata & Kariya, 2010; Uetake *et al.*, 2011; Cafazzo *et al*., 2012; Prevedello *et al*., 2012; Webb *et al*., 2012; Abdelfattah *et al*., 2013; Fisher *et al.*, 2014; Jongman & Butler, 2014; Llonch *et al*., 2015
Drink/Chew/ruminate	49 (19.4)	de Wilt, 1985; Kent & Ewbank, 1986a; Veissier *et al.,* 1989; Dellmeier *et al*., 1990; Lidfors, 1993; Rushen & de Passillé, 1995; Jarvis *et al*., 1996; Fisher *et al*., 1997; Molony & Kent, 1997; Veissier *et al.,* 1997; Veissier *et al*., 1998; Cockram *et al*., 1999; Grasso *et al.,* 1999; Knowles *et al.,* 1999; Munksgaard *et al*., 1999; Morisse *et al.,* 2000; Bokkers & Koene, 2001; Fregonesi & Leaver, 2001; Grigor *et al*., 2001; Haley *et al*., 2001; Gottardo *et al.*, 2002; Ibáñez *et al.,* 2002; Mattiello *et al*., 2002; Margerison *et al.,* 2003; Grigor *et al.*, 2004; Herskin *et al.*, 2004; Panivivat *et al.,* 2004; Brown-Brandl *et al.*, 2005; Raussi, 2005; Miller *et al*., 2006; Tapkı *et al.,* 2006; Schwartzkopf-Genswein *et al*., 2007; Budzynska & Weary, 2008; Candiani *et al*., 2008; Jasper *et al*., 2008; Pritchard *et al.,* 2008; Webster *et al*., 2008; Bokkers *et al*., 2009; Reefmann *et al.,* 2009; Westerath *et al*., 2009; Ishiwata & Kariya, 2010; Cafazzo *et al.*, 2012; Duve *et*

Welfare Indicator	Number of Studies (%) n=253	Reference
		al., 2012; Prevedello *et al.,* 2012; Webb *et al.*, 2012; Abdelfattah *et al*., 2013; Jongman & Butler, 2014; Peli *et al.,* 2016
Standing	46 (18.2)	Blaxter & Wood, 1951; de Wilt, 1985; Veissier *et al.,* 1989; Vermorel *et al.*, 1989; Dellmeier *et al.*, 1990; Mellor *et al*., 1991; Schrama *et al.*, 1993a; Hopster & Blokhuis, 1994; Stull & McDonough, 1994; Schrama *et al.,* 1995; Jarvis *et al.*, 1996; Jensen *et al.*, 1997; Knowles *et al.,* 1997; Todd, 1998; Grasso *et al*., 1999; Knowles *et al*., 1999; Munksgaard *et al.*, 1999; Haley *et al.*, 2000; Boissy *et al*., 2001; Fregonesi & Leaver, 2001; Haley *et al.*, 2001; Veissier *et al.*, 2001; Ibáñez *et al*., 2002; Mattiello *et al.*, 2002; Margerison *et al.,* 2003; Mülleder *et al.,* 2003; Van de Water *et al.*, 2003a; Waiblinger & Menke, 2003; Grigor *et al*., 2004; Herskin *et al.,* 2004; Panivivat *et al.*, 2004; Regula *et al*., 2004; Waiblinger *et al.,* 2004; Garner, 2005; Raussi, 2005; Færevik *et al.*, 2006; Tapkı *et al.*, 2006; Schwartzkopf-Genswein *et al.*, 2007; Budzynska & Weary, 2008; Candiani *et al.*, 2008; Webster *et al.*, 2008; Earley & Murray, 2010; Ishiwata & Kariya, 2010; Uetake *et al.*, 2011; Cafazzo *et al*., 2012; Webb *et al.,* 2012
Movement	39 (15.4)	Dellmeier *et al.*, 1990; Tarrant *et al.,* 1992; Hopster & Blokhuis, 1994; de Passillé *et al*., 1995; Jarvis *et al*., 1996; Jensen *et al.,* 1997; Veissier *et al*., 1997; Veissier *et al.*, 1998; Veissier *et al.*, 1989; Grasso *et al*., 1999; Jensen, 1999; Jensen & Kyhn, 2000; Boissy *et al.*, 2001; Jensen, 2001; Lensink *et al.*, 2001a; Veissier *et al.,* 2001; Ibáñez *et al.*, 2002; Margerison *et al*., 2003; Van de Water *et al.,* 2003b; Grigor *et al.,* 2004; Van Reenan *et al.*, 2004; Waiblinger *et al.,* 2004; Napolitano *et al.*, 2005; Raussi, 2005; Van Reenen *et al.*, 2005; Færevik *et al.,* 2006; Miller *et al.,* 2006; Tapkı *et al.,* 2006; Schwartzkopf-Genswein *et al.,* 2007; Jasper *et al.,* 2008; Sandström, 2009; Ishiwata & Kariya, 2010; Krachun *et al*., 2010; Bourguet *et al.*, 2011; Uetake *et al.,* 2011; Duve *et al*., 2012; Weschenfelder *et al.*, 2012; Hultgren *et al.,* 2014; Lürzel *et al.,* 2015b

Welfare Indicator	Number of Studies (%) n=253	Reference
Oral	33 (13.0)	Blaxter & Wood, 1951; de Wilt, 1985; Wiepkema *et al*., 1987; Veissier *et al.,* 1989; Dellmeier *et al.,* 1990; Kooijman *et al.*, 1991; Lidfors, 1993; Rushen & de Passillé, 1995; Molony & Kent, 1997; Veissier *et al.*, 1997; Veissier *et al*., 1998; Munksgaard *et al.,* 1999; Bokkers & Koene, 2001; Gottardo *et al*., 2002; Mattiello *et al*., 2002; Margerison *et al*., 2003; Mülleder *et al.,* 2003; Van de Water *et al.,* 2003a; Panivivat *et al*., 2004; Garner, 2005; Raussi, 2005; Smulders *et al*., 2006; Jasper *et al.*, 2008; Bokkers *et al.*, 2009; Ishiwata & Kariya, 2010; Brščić *et al.*, 2012; Cafazzo *et al.*, 2012; Duve *et al.*, 2012; Miranda de la Larna *et al*., 2012; Webb *et al*., 2012; Abdelfattah *et al*., 2013; Leruste *et al.*, 2014; Lürzel *et al.,* 2015a
Vocalising	25 (9.9)	Willet & Erb, 1972 ; Dellmeier *et al.*, 1990; Lay *et al*., 1992; de Passillé *et al.,* 1995; Jensen *et al.*, 1997; Grandin, 1998a; Grandin, 1998b; Schrader & Todt, 1998; Watts & Stookey, 2000; Grandin, 2001; Van de Water *et al.*, 2003a; Maria *et al.,* 2004; Van Reenan *et al.,* 2004; Napolitano *et al.,* 2005; Van Reenen *et al.*, 2005; Færevik *et al.,* 2006; Lauber *et al.*, 2006; Budzynska & Weary, 2008; Jasper *et al.*, 2008; Dalmau *et al.,* 2009; Sandström, 2009; Bourguet *et al.,* 2010; Hemsworth *et al*., 2011; Weschenfelder *et al.*, 2012; Hultgren *et al*., 2014
Animal Handling	22 (8.7)	Grandin, 1998a; Grandin, 1998b; Veissier *et al.,* 1998; Jago *et al.*, 1999; Lensink *et al.,* 2000a; Lensink *et al.*, 2000b; Bokkers & Koene, 2001; Lensink *et al.,* 2001a; Lensink *et al.,* 2001b; Lensink *et al.*, 2003; Regula *et al.,* 2004; Van Reenen *et al.,* 2004; Garner, 2005; Rousing *et al.*, 2005; Hemsworth *et al.*, 2011; González *et al.*, 2012b; Leruste *et al.,* 2012b; Schütz *et al.*, 2012; Hultgren *et al*., 2014; Stanković *et al*., 2014; Lürzel *et al.,* 2015a; Lürzel *et al*., 2015b
Eliminating	20 (7.9)	Kertz *et al.*, 1984; Kenny & Tarrant, 1987; Schrama, 1992; Schrama *et al.,* 1993; de Passillé *et al.*, 1995; Jensen *et al.*, 1997; Jago *et al.*, 1999; Lensink *et al.,* 2000b; Parker *et al.*, 2003; Van de Water *et al*., 2003a; Van de Water *et al.,* 2003b; Maria *et al.,* 2004; Napolitano *et al.,* 2005; Van Reenen *et al.,* 2005; Lauber *et al.,* 2006; Smulders *et al.*, 2006; Tapkı *et al.,* 2006; Ishiwata *et al.*, 2008; Hultgren *et al.,* 2014; Lürzel *et al.,*

Welfare Indicator	Number of Studies (%) n=253	Reference
		2015b
Sniffing/Licking	20 (7.9)	de Wilt, 1985; Tarrant *et al.,* 1988; de Passillé *et al*., 1995; Jensen *et al., 1*997; Veissier *et al.*, 1997; Veissier *et al.,* 1998; Jensen, 1999; Jensen & Kyhn, 2000; Boissy *et al.*, 2001; Jensen *et al.,* 2001; Veissier *et al.*, 2001; Mattiello *et al.,* 2002; Herskin *et al.*, 2004; Raussi, 2005; Lauber *et al.,* 2006; Sandström, 2009; Westerath *et al*., 2009; Duve *et al.*, 2012; Miranda de la Larna *et al.,* 2012; Webb *et al.*, 2012
Grooming	19 (7.5)	Dellmeier *et al.*, 1990; Molony & Kent, 1997; Veissier *et al.,* 1997; Veissier *et al.*, 1998; Grasso *et al.*, 1999; Munksgaard *et al.*, 1999; Bokkers & Koene, 2001; Mattiello *et al.*, 2002; Margerison *et al.,* 2003; Herskin *et al.,* 2004; Panivivat *et al.*, 2004; Raussi, 2005; Bokkers *et al.*, 2009; Ishiwata & Kariya, 2010; Miranda de la Larna *et al.,* 2012; Schütz *et al.*, 2012; Webb *et al.*, 2012; Abdelfattah *et al.*, 2013; Lürzel *et al.,* 2015b
Playing	17 (6.7)	Blaxter & Wood, 1951; Kenny & Tarrant, 1987; Jensen *et al*., 1998; Jago *et al*., 1999; Jensen & Kyhn, 2000; Jensen *et al.,* 2001; Mülleder *et al*., 2003; Smulders *et al*., 2006; Tapkı *et al.,* 2006; Ishiwata & Kariya, 2010; Krachun *et al*., 2010; Duve *et al*., 2012; Schütz *et al.*, 2012; Abdelfattah *et al.*, 2013; Mintline *et al*., 2013; Llonch *et al.,* 2015; Lürzel *et al*., 2015a
Exploration	15 (5.9)	Kenny & Tarrant, 1987; Tarrant *et al.,* 1988; Dellmeier *et al.*, 1990; Tarrant *et al.*, 1992; Veissier *et al.*, 2001; Panivivat *et al.*, 2004; Færevik *et al.*, 2006; Lauber *et al.*, 2006; Candiani *et al.,* 2008; Westerath *et al.*, 2009; Ishiwata & Kariya, 2010; Cafazzo *et al.,* 2012; Schütz *et al.,* 2012; Hultgren *et al.*, 2014; Lürzel *et al.,* 2015b
Idle	15 (5.9)	Veissier *et al*., 1989; de Passillé *et al.,* 1995; Molony & Kent, 1997; Veissier *et al.,* 1997; Veissier *et al.*, 1998; Grasso *et al.,* 1999; Jago *et al.,* 1999; Munksgaard *et al.*, 1999; Jensen *et al.,* 2001; Huxley *et al.*, 2004; Waiblinger *et al.*, 2004; Raussi, 2005; Bokkers *et al.,* 2009; Sandström, 2009; Hultgren *et al*., 2014

Welfare Indicator	Number of Studies (%) n=253	Reference
Mounting	15 (5.9)	Kenny & Tarrant, 1987; Tarrant *et al.*, 1988; Dellmeier *et al*., 1990; Jarvis *et al.*, 1995; Jarvis *et al*., 1996; Veissier *et al.*, 1998; Jensen *et al.,* 2001; Van de Water *et al.,* 2003b; Grigor *et al.*, 2004; Maria *et al.*, 2004; Bokkers *et al.*, 2009; Sandström, 2009; Duve *et al.,* 2012; Miranda de la Larna *et al*., 2012; Webb *et al*., 2012
Slipping/falling	15 (5.9)	Tarrant *et al.*, 1988; Dellmeier *et al.,* 1990; Tarrant *et al.*, 1992; Jarvis *et al.,* 1995; Jarvis *et al.*, 1996; Grandin, 1998a; Jacobson & Cook, 1998; Van de Water *et al.*, 2003b; Grigor *et al.*, 2004; Maria *et al.,* 2004; Bokkers *et al.,* 2009; Dalmau *et al.*, 2009; Sandström, 2009; Weschenfelder *et al*., 2012; Hultgren *et al*., 2014
Aggressive	14 (5.5)	Tarrant *et al.,* 1988; Tarrant *et al*., 1992; Fisher *et al.,* 1997; Grasso *et al.,* 1999; Veissier *et al.*, 2001; Margerison *et al.*, 2003; Raussi, 2005; Smulders *et al.,* 2006; Candiani *et al.*, 2008; Sandström, 2009; Cafazzo *et al.*, 2012; Miranda de la Larna *et al.,* 2012; Abdelfattah *et al.,* 2013; Llonch *et al.*, 2015
Butting	12 (4.7)	de Wilt, 1985; Kenny & Tarrant, 1987; Tarrant *et al.,* 1988; Jarvis *et al.,* 1995; Rushen & de Passillé, 1995; Jarvis *et al.*, 1996; Veissier *et al.*, 1998; Jago *et al.,* 1999; Fregonesi & Leaver, 2001; Jensen *et al.,* 2001; Mülleder *et al.*, 2003; Waiblinger *et al.*, 2004; Raussi, 2005; Bokkers *et al.,* 2009; Duve *et al.,* 2012; Miranda de la Larna *et al.*, 2012; Webb *et al.*, 2012
Kicking	10 (4.0)	Molony & Kent, 1997; Jensen *et al.,* 2001; Lensink *et al*., 2001; Lensink *et al.*, 2001; Waiblinger *et al.,* 2004; Napolitano *et al.,* 2005; Bourguet *et al*., 2010; Miranda de la Larna *et al.*, 2012; Webb *et al.*, 2012; Hultgren *et al.,* 2014
Social	10 (4.0)	Fisher *et al.*, 1997; Veissier *et al.*, 1998; Grasso *et al.,* 1999; Bokkers & Koene, 2001; Veissier *et al.,* 2001; Mattiello *et al.,* 2002; Mülleder *et al.*, 2003; Færevik *et al.*, 2006; Duve *et al.*, 2012; Miranda de la Larna *et al.,* 2012
Avoidance	9 (3.6)	Lay *et al.,* 1992; Mülleder *et al.,* 2003; Waiblinger & Menke, 2003; Waiblinger *et al.*, 2004; Napolitano *et al.*, 2005; Windschnurer *et al*., 2008; Schütz *et al.,* 2012; Lürzel *et*

Welfare Indicator	Number of Studies (%) n=253	Reference
		al., 2015a; Lürzel *et al.,* 2015b
Qualitative Behaviour Assessment	9 (3.6)	Wemelsfelder *et al.*, 2000; Stockman *et al.,* 2011; Brščić *et al*., 2012; Stockman *et al.,* 2012; Andreasen *et al.*, 2013; Stockman *et al*., 2013; Ellingsen *et al.,* 2014; Llonch *et al.,* 2015; Wickham *et al.*, 2015
Resting	9 (3.6)	Mogensen *et al.*, 1997; Molony & Kent, 1997; Munksgaard *et al.*, 1999; Hänninen *et al.,* 2003; Zähner *et al*., 2004; Hänninen *et al.*, 2005; Tapkı *et al.*, 2006; Ishiwata *et al.*, 2008; Dalmau *et al.*, 2009
Jump	7 (2.8)	Dellmeier *et al.*, 1990; de Passillé *et al.*, 1995; Maria *et al.*, 2004; Sandström, 2009; Krachun *et al.,* 2010; Duve *et al.*, 2012; Webb *et al.*, 2012
Active	6 (2.4)	Blaxter & Wood, 1951; Vermorel *et al.,* 1983; Vermorel *et al.*, 1989; Molony & Kent, 1997; Veissier *et al.*, 2001; Bokkers *et al.,* 2009
Agonistic	6 (2.4)	Kenny & Tarrant, 1987; Tarrant *et al.,* 1988; Fregonesi & Leaver, 2001; Mülleder *et al.,* 2003; Waiblinger *et al.*, 2004; Raussi, 2005
Balk	5 (2.0)	Jago *et al.,* 1999; Van de Water *et al.,* 2003b; Maria *et al.*, 2004; Sandström, 2009; Weschenfelder *et al.*, 2012
Rubbing	5 (2.0)	de Wilt, 1985; Ishiwata *et al.,* 2008; Westerath *et al.,* 2009; Miranda de la Larna *et al.*, 2012; Webb *et al.*, 2012
Withdrawal distance	5 (2.0)	Lensink *et al.,* 2000b; Lensink *et al.,* 2001a; Mülleder *et al.,* 2003; Rousing *et al.*, 2005; Lauber *et al.*, 2006
Stereotypies	5 (2.0)	Wiepkema *et al.,* 1987; Maria *et al*., 2004; Smulders *et al*., 2006; Webb *et al*., 2012; Llonch *et al.,* 2015
Scratching	4 (1.6)	de Wilt, 1985; Veissier *et al.,* 1997; Bokkers *et al*., 2009; Miranda de la Larna *et al.,* 2012

Welfare Indicator	Number of Studies (%) n=253	Reference
Turn	3 (1.2)	Sandström, 2009; Webb *et al*., 2012
Escape	2 (0.8)	Lay *et al*., 1992; Lürzel *et al.,* 2015b
Head shaking	2 (0.8)	Waiblinger *et al*., 2004; Webb *et a*l., 2012
Nose licking	2 (0.8)	de Wilt, 1985; Bokkers *et al.,* 2009
Stretching	2 (0.8)	de Wilt, 1985; Bokkers *et al*., 2009
Struggling	2 (0.8)	Duve *et al*., 2012; Hultgren *et al*., 2014
Comfort behaviour	1 (0.4)	Bokkers & Koene, 2001
Compression	1 (0.4)	Bokkers *et al.,* 2009
Crowding	1 (0.4)	Hultgren *et al.,* 2014
Head lifting	1 (0.4)	Bourguet *et al.,* 2010
Learning	1 (0.4)	Lauber *et al.*, 2006
Loading/Unloading time	1 (0.9)	Maria *et al.*, 2004
Non-agonistic	1 (0.4)	Raussi, 2005
Positive	1 (0.4)	Llonch *et al.*, 2015
Return time	1 (0.4)	Lauber *et al.,* 2006
Tail flicking	1 (0.4)	Lürzel *et al*., 2015b
Vigour	1 (0.4)	Todd, 1998

2.5 Discussion

The systematic mapping of the literature has provided a structured and comprehensible recognition of the primary characteristics of calf welfare from global research publications. The bobby calf industry is particularly relevant to New Zealand and Australia; the majority of the 253 studies identified were conducted in Europe, the United States and New Zealand. The search terms allowed for the identification of articles relating to bobby calves, however, inclusion criteria were not restricted and allowed for other neonatal animals and veal calves that are typically slaughtered at an older age. While neonates of other species may have different experiences compared to bobby calves; the studies helped to identify a range of potential welfare indicators that can then be assessed for validity, practicality, reliability and feasibility for use in commercial settings for New Zealand bobby calves.

Gaining an international perspective on indicators used to evaluate welfare compromise and enhancement in young calves is valuable to better understand the published research and allows similarities and differences in the approaches to welfare evaluation to be compared across different countries. The geographical distribution of the search results plausibly represents production systems from industrialised regions where animal welfare science has been topical for many years (Fraser, 2008). However, the geographic distribution of research papers may also be due to the language restrictions placed on the acceptance of articles retrieved. The distribution of the articles agrees with other publications (e.g. Fraser, 2008; Fakoya, 2011) which indicated that, primarily in industrialised nations, the increase in production of meat from the 1960s has coincided with debates over the ethical treatment of animals and advances in science which refined and clarified the welfare issues (Fraser, 2008).

The research question was to identify potential indicators of neonatal animal welfare; however, the diversity of the returned publications meant that results were not limited to neonates and studies involving welfare indicators in animals of numerous ages and types were returned. While, ultimately, the purpose of mapping this literature was to compile a comprehensive list of welfare indicators, the selection of publications related to welfare assessed in a diverse range of facilities, including on farm, in commercial calving and veal systems, during transport, and at abattoirs. Accepting such articles allowed identification of a wider range of welfare indicators that may be valid and practical for assessment of bobby calf welfare.

Although mapping of the literature identified numerous indicators used to assess welfare in past studies, this exercise did not evaluate the validity, reliability, repeatability and feasibility of the indicators. Previous work has been done to attempt to assess the validity of welfare indicators. For example, an investigation into the validity of various indicators used to evaluate sheep welfare at abattoirs was undertaken by Llonch and colleagues (2015). However, 'validity' in this evaluation was based upon reports of validity in the studies in which the indicator was originally used. More recent work to validate potential indicators of sheep welfare has been completed by Beausoleil & Mellor (2017). The authors suggest that rigorous validation requires evidence of the links between the indicator (e.g. behaviours observed) and physiological state (e.g. hydration status) and between the physiological state and the particular mental experiences that influence welfare status (e.g. thirst). Further research to investigate the validity of specific calf welfare indicators needs to be undertaken to provide a useful list of indicators that will encourage more detailed and holistic assessments in commercial facilities such as abattoirs or during transportation.

The research publications identified in this review demonstrate an increasing trend in animal welfare research since the 1990s. This trend coincides with the increasing focus on animal welfare in livestock production systems, which has occurred over the last two decades (Tosi et al., 2001; Sejian *et al.*, 2011). In Europe, existing welfare assessment systems focus on management and housing (Sejian *et al.*, 2011), however, there is increasing consideration of more parameters of animal welfare (Sejian, 2007; Sejian *et al.*, 2011). Consequently, health and behavioural indicators have arisen, making welfare assessments more comprehensive (Albright, 1983; Broom, 1991). The understanding of the mental and physical dimensions of animal's welfare supports advances in understanding and evaluating animal welfare (Mellor & Webster, 2013).

Given the search terms used, it is not suprising that the majority of the studies gathered during the retrieval process were conducted on cows (87%). That said, when the search terms returned relevant articles involving other species they were included so that as many potentially relevant indicators could be retrieved. There is limited research regarding indicators of welfare in bobby calves, hence the inclusion of other species allows a more comprehensive set of potential welfare indicators to be returned. Articles were not excluded because of the species in which the work was conducted unless the work was considered as irrelevant. The inclusion of other species potentially weakens the accuracy and applicability of some of the indicators identified to be relevant to bobby calves in the current mapping. There may be other welfare indicators discussed in different species, for example companion animals, that are not detected or addressed in the articles that were screened during the search. Likewise, a limitation of the search terms of the current mapping review is that it may have failed to identify welfare indicators that are potentially relevant for neonatal animals but have only been studied in adult animals. Articles were not excluded because of the age of the species in which the work was conducted unless the work was deemed irrelevant. The search terms used are primarily focussed on returning animal-based welfare indicators, however, resource-based indicators were also retrieved. This is a limitation of the search terms, as relevant resource-based welfare indicators may have been missed in the search. Originally, the term farm was also included in the search terms, however, this returned a formidable number of articles. Therefore, the decision was made to remove the term and focus on those related to transport and slaughter as they were more relevant to the research question.

The consistency of nomenclature and descriptions of the welfare indicators varied between papers. When the terms used were almost identical then they were merged. For example, the behavioural indicator 'playing' is referred to as "play" in 17 of 253 articles. However, if entirely different words even if the meaning was similar or if the authors focused on a specific feature we did not merge the terms. For example, other studies record different aspects of play, such as locomotor play, social play or object play. An example of locomotor play is jumping, which was recognised in 7 further articles. Categorisation of behaviours is partially dependant on the definition given of the specific behaviour which is why the potential behavioural indicators are presented as they were recorded in each article and reorganisation of behavioural indicators of welfare in Table 2. 6 has not occurred.

During the search process, the screening of the returned articles included primarily quantitative research: experimental studies, obervational studies and systematic reviews, but rejected narrative literature reviews and reports not based around experimental data. A potential limitation of the use of the search engines is missing relevant literature that is either unpublished or in the form of a report. The selection of the four search engines was discussed with librarians at Massey University who specialise in literature researching. Scopus, Discover, Web of Science and Google Scholar were selected due to the relevance of the articles returned in a pilot screening. The search terms were adjusted numerous times between June 2016 and Janurary 2017, with the resulting terms returning a manageable number of relevant articles. Google Scholar, also included as a search engine, returned a high number of articles with the lowest percentage accuracy. The majority of Goggle Scholar retrieved articles were also returned from another search engine. It is interesting that searching of the references returned more relevant articles than the initial search term. This further illustrates the limitations of the search terms used.

Although the resulting literature comprised scientific experiments and observational studies, there is a concern about the accuracy of the results in some publications due to the small sample size used. For example, Locatelli and colleagues (1989) looked at the adrenal response to simulated transport in 3 calves. While the experiment was conducted in order to assess blood constituents as indicators of stress, the use of such a small sample size carries a high risk of misinterpretation of the results.

The primary limitation in conducting a mapping of the literature is human error. The amount of literature returned in the search was extensive, and ensuring all data were accurately recognised and entered into the database was essential. The 253 relevant articles addressed in this systematic mapping were reviewed numerous times in order to collect results and gain a better understanding of the literature.

2.6 Conclusion

Systematic mapping is a method allowing the broad range of literature available in a particular field to be analysed and is useful for evidence-based decision making (Arskey & O'Malley, 2005). The current systematic mapping review provides constructive information useful for directing future research involving assessment of the welfare of young calves. The research identified in this review addressed a variety of potential animal-based and resource-based welfare indicators that could be useful for assessing young calves in commercial abattoirs. A total of 99 potential nutritional, environmental, health and behavioural indicators were recognised from 253 relevant articles. The following study, presented in Chapter 3, used a selection of the potential welfare indicators identified in this systematic mapping to assess bobby calf welfare in lairage facilities across New Zealand.

Chapter 3: Descriptive analysis of the cross-sectional study of the prevalence of potential welfare indicators in New Zealand bobby calves

3.1 Introduction

Animal welfare assessment aims to identify areas of potential compromise so actions can be taken to reduce or mitigate the cause of compromise in order to safeguard the animal (Blokhuis *et al.,* 2003). This is particularly important in bobby calves because they are transported for slaughter at a young age, typically between four to seven days old, and are subjected to potentially stressful events. Historically bobby calf welfare in New Zealand has focussed on mortality as an indicator, and current regulations are aimed at reducing mortality or condemnation following transport or in lairage for welfare reasons.

The bobby calf industry is highly regulated through enforceable standards developed according to the 2015 amendment of the Animal Welfare Act 1999. Recent additions to such policies, through the Animal Welfare (Calves) Regulations 2016, include the development of regulations which cover specific areas of non-compliance believed to increase the risk of compromised animal welfare. Bobby calves transported from the farm to the processing plant must have firm hooves and a dry navel (MPI, 2014). Furthermore, regulated requirements introduced on 1st August 2016 mean it is statutory for all bobby calves transported for slaughter to be: 1) at least four days old; 2) healthy with a bright and alert demeanour; 3) strong enough to stand, move around and walk onto the transport truck without assistance; and 4) travel duration must not exceed 12 hours (MPI, 2016). In addition, effective from 1st February 2017, calves should not be denied access to feed for more than 24 hours (previously it was 30 hours) and maximum time in lairage is 20 hours (MPI, 2014; MPI, 2016). Whilst some of these changes took place during the 2016 bobby calf season in which this study was conducted, the regulations introduced in 2017 were not enforceable during this time.

Despite the new statutory requirements, some calves still do not survive the journey or are so seriously compromised they need be euthanised before they can be slaughtered. In 2008, the calf mortality rate (deaths and condemnations) was

0.68% (MPI, 2017). Efforts by a range of parties involved in the dairy industry have contributed to the significant decrease in mortality rate (0.12% in 2016). Low mortality rates have been achieved through improved education on rearing and handling of calves as well as voluntary compliance with the regulations in place (MPI, 2017). Nonetheless, members of the public find it concerning that more than 2000 calves still died in 2016 before the point of slaughter (MPI, 2017), although the mortality rate of 0.12% can be considered as very low (MPI, 2017).

Previous work has identified that the length of the journey and time in lairage are factors which influence calf mortality (O'Grady & Thomas, 2013). In addition, risk of mortality increases during the bobby calf season (O’Grady & Thomas, 2013). This could be a result of the meat processing plant slaughter schedule changing towards the end of the processing season, or of the accumulation of pathogens in calf sheds on farm (O’Grady & Thomas, 2013).

Despite the low mortality rate, there is still opportunity to improve the individual animals’ experiences and welfare. Currently, the only recorded parameters of welfare are mortality rate and the findings from post-mortem examinations which are completed on those animals that either die or are condemned before slaughter by the veterinary staff responsible at each meat processing plant. The use of calf mortality alone is inadequate for welfare assessment because opportunities for welfare compromise occur throughout the supply chain (Ortiz-Pelaez *et al.,* 2008). Rather a more holistic approach to assessing bobby calf welfare is required so the causes of poor welfare from the public perspective, farmers and industry bodies can be identified and improved procedures be implemented.

Throughout the supply chain, opportunities for welfare compromise occur. Identifying areas of potential compromise facilitates the identification of the risk factors and allows improvements to be made to calf welfare. However, there have been few studies either internationally or in New Zealand that address this. This study aimed to firstly determine the prevalence of potential animal-based ante-mortem indicators of poor calf health and welfare at the processing plant in lairage prior to slaughter. The second aim was to evaluate the effect of time in study and time in lairage on the percentage of calves showing selected indicators.

3.2 Materials and Methods

Massey University Animal Ethics Committee approved all procedures for this study (Protocol 16/56).

3.2.1 *Animals and study design*

From 28th June to 25th October 2016 a cross-sectional study was undertaken observing bobby calves in lairage at twelve different meat processing plants across New Zealand. Plants were selected for inclusion based on three criteria. First, they were required to be in the region the researchers were visiting in a particular week for a larger scale case-control study being run at the same time. The second criterion was that the slaughter schedule for the processing plants in the region would allow for observations to be conducted. Specifically, it was required that the calves would have been in lairage for at least one hour before observations began, allowing time for calves to settle, and that observations would not be disrupted by yard staff or vets wanting to take the animals up to the slaughter chain. The third condition was that at least three consignments of calves were expected to be arriving at the plant on the day planned for sampling. Sampling was not conducted on a consistent day of the week or at a regular time as the slaughtering schedule differed among the meat processing plants.

Data were collected from twelve meat processing plants, with data collected at six of these plants more than once during the study period (Table 3.1). The plants were located in eight regions over the North Island and South Island of New Zealand. The number of pens observed at each plant varied depending on the number of consignments that had arrived that particular day and the plant’s slaughter schedule (Table 3.1). For confidentiality, the meat processing plants visited during the study have been given a random number and have not been identified. All observations were carried out by one investigator who has past experience working with dairy calves.

Bobby calves were initially assessed as groups in pens at lairage. The number of calves observed in the pens at the group level varied according to the sizes of the consignments that had arrived at the plant that day. Observations were collected using recording forms as presented in Appendix A (Table A1) for group level and Appendix A (Table A2) for individual level.

3.2.2 *Collection of group level data*

The first part of the study involved field observations of groups of bobby calves in lairage at the meat processing plants that had been in lairage for at least one hour before the start of observations, allowing a period for the calves to 'settle' after arrival. The pens to be observed were randomly selected. This requirement often dictated the order in which the pens were observed. If numerous pens of calves had been in lairage for longer than the one hour settling period, the pens were given a number and a random number was selected from a random number table. The observations and measurements described below were made systematically using a recording form (Appendix A).

Time in lairage was determined using the arrival time until the start of observations using records provided at the meat processing plants and noting the time that observations of that particular pen of animals started. Records were either in the form of a truck docket which provided arrival time for consignment and therefore the pen of calves, or yards men records on which truck dockets had already been transcribed into the required paperwork. The week of the study is a continuous variable and is the week in which the observations were made for a particular meat processing plant and represents time in the spring calving season but is roughly a proxy for the time in season (e.g. early in season: week 1-6, middle of season: week 7-12, end of season: week 13-18).

Observations were first made from outside the pen, without disturbing the animals. Binoculars were used to observe the calves more closely when necessary. The number of calves in the pen was first recorded. The group was then observed for two minutes and the number of animals exhibiting certain behaviours was determined. Similarly the number of animals in the pen displaying a number of health indicators was determined. A full list of the behaviours and health indicators assessed, along with definitions, can be found in Table 3.2. After all the observations were made (group and individual level – see Section 3.2.3) the outside of the pen was measured using a tape measure so the stocking density could be determined.

After the undisturbed observations were made from outside the pen, a systematic walk through the pen was completed for every pen where at least one calf was lying. The walk through was done in the same way for each pen: the researcher began at one corner and crossed the pen diagonally at a constant pace. She then followed the fence line to the next corner and crossed diagonally the other way before following the fence line to the final corner. If calves were sleeping in the path of the walk through, the researcher walked around the calves while keeping as close as possible to the plan. After the walk through, calves that were still lying were approached directly towards their head to a distance of 0.5 metres and it was noted whether they stood or not. At each of the following stages the number of calves standing was recorded: 1) before entering pen; 2) upon pen entry; 3) during the walk through; 4) during direct approach and 5) five minutes after walk through was completed.

3.2.3 *Collection of Individual animal data*

After the group observation was complete, a subsample of five calves in each pen was randomly selected using a simple random sampling design for assessment as individuals for potential indicators of poor welfare status (Table 3.2).

Random selection of five calves in a pen was achieved using a random number table; the researcher would start counting calves (in the same pattern as the systematic walk-through) and the supervisor (from outside the pen) would notify the researcher when the random numbered calf was reached. A strip of duct tape was attached to the calf's back to identify it for assessment to be undertaken once all five had been selected. The researcher would then begin counting calves again from that point. The supervisor would use the next random number in the table to notify the researcher when the next random numbered calf was reached. The process was repeated until 5 calves were selected.

Once the five calves were selected, behavioural observations were made, this time from within the pen, but with as little disturbance to the calves as possible. The aim was to observe calves from greater than one metre away, however, the calf's behaviours, posture (lying or standing), the pen stocking density, and behaviours of other calves influenced the distance from which calves were observed. The sex and breed of each calf was recorded. Each individual was observed for two minutes looking for a range of behaviours followed by recording the presence or absence of the indicators of health described in Table 3.2.

Respiratory rate was measured over a twenty second period and then multiplied by three to calculate the animal's respiration rate in breaths per minutes. This variable was categorised into decreased (<24 breaths per minute; BPM), normal (24-36 BPM) or increased (>36 BPM) (after: Jackson & Cockcroft, 2002).

Finally, a skin tent test was performed on each of the randomly selected calves. When conducting the test the calves remained in the posture they were assessed in, either standing or lying, and were gently positioned with their head up and facing straight ahead. The researcher's right hand was placed with the lateral edge resting lightly against the calf's scapula. A firm pinch of skin was taken between thumb and index finger, cranial to the cranial border of the scapula on the calf's neck. This fold of pinched skin was immediately released. A stop watch was activated at the time of release of the skin fold. Timing was stopped when the skin had returned to the flattened position and was no longer moving. Calves were classified as having moderate dehydration when the skin taking two or more seconds to return to normal after tenting (after: Constable et al., 1998). Due to contradicting information in the literature, moderate dehydration was also defined as the skin taking three or more seconds to return to normal after tenting (after: Walker *et al.,* 1998).

3.3 Statistical Analysis

The outcome of interest in this study was the prevalence of the potential welfare indicators described in Table 3.2. The analysis was conducted in R version 0.98.932 (R Development Core Team, 2014). The level of significance was set at $P<0.05$.

The data consisted of group level data and individual animal data. The variables in the group data set were recorded as the percentage of calves in the pen that were observed demonstrating the behaviour. For each of the prevalent potential welfare indicators, the percentage of calves observed to be doing the behaviour was non-normally distributed. Therefore, the distributions are summarised using minimum, maximum, median and quartiles.

The individual calf data comprised of twelve categorical variables and two continuous variables, respiration rate and skin fold test. Histograms were generated to describe the respiration rate and skin tent test. The results of the skin fold test were used to create two different categorical variables. Similarly, respiration rate was initially recorded as a continuous variable but was modified to a categorical variable as either increased respiratory rate of greater than 36 breaths per minute ('yes') or not increased respiratory rate of less than or equal to

36 breaths per minutes (‘no’). Categorical variables were reported as counts and percentage in each category.

Group or individual outcomes observed in greater than or equal to 20% of the calves were analysed further in a two-step process. The first step was to determine if any of the measured outcomes were significantly associated with either time in lairage or week in study. In order to assess significance Kruskal-Wallis test was used which had either lairage or week in study as the outcome variable and the group or individual outcome as the predictor. The second step involved construction of a mixed effects model logistic regression to assess the significance of time in lairage and week in study simultaneously with a random effect to account for clustering within regions.

Table 3.1: Schedule of visits to processing plants over the 18 week period in 2016 along with the number of pens sampled on each visit

Date	Plant Number	Pens sampled (number)
28/06/2016	1	2
5/07/2016	2	6
6/07/2016	2	2
12/07/2016	3	3
13/07/2016	3	2
22/07/2016	4	1
25/07/2016	5	1
28/07/2016	6	2
29/07/2016	6	3
1/08/2016	7	4
8/08/2016	1	6
15/08/2016	2	6
22/08/2016	8	7
29/08/2016	9	6
5/09/2016	6	4
6/09/2016	6	3
12/09/2016	7	6
19/09/2016	1	6
26/09/2016	1	6
5/10/2016	8	7
10/10/2016	10	6
19/10/2016	11	6
25/10/2016	12	7

Table 3.2: Description of potential welfare indicators assessed in bobby calves in lairage facilities at 12 meat processing plants across New Zealand during the cross-sectional study

Indicator	Description	Level Assessed
Coughing	The rapid and noisy expulsion of air from the lungs. Recorded as either being present or absent.	G & I_1
Faecal Soiling	The presence of faecal material around the anus, hindquarters and/or hind legs. Recorded as either being present or absent.	G & I
Head Shaking	Repeated rapid movement of the head either side to side, up and down or a combination of both. Recorded as being present or absent	G & I
Head Tilting	Head tilted to one side observed in calves either standing or in sternal recumbency. Recorded as being either present or absent.	G & I
Huddling	The action of calves standing or lying in close proximity of other animals where at least 50% of its body is in contact with another animal. Recorded as being either present or absent	G
Injury	The presence of hairless patches, swellings or lesions. Calves were given an injury score according to Table 3.3.	G & I
Lying	Position of recumbency while the animals are undisturbed. Noted whether present or absent (standing).	G & I
Nasal Discharge	Discharge from the nostril(s). Noted as: present or absent, unilateral or bilateral, and serous or mucopulurent.	G & I
Ocular Discharge	Discharge from the eye. Noted as: present or absent, unilateral or bilateral, and serous or mucopulurent.	G & I

Indicator	Description	Level Assessed
Oral Behaviours	The expression of non-nutritive oral activities. Noted while the calves are undisturbed as being absent or present. The focus of the behaviour was also recorded as: manipulating an object, cross-sucking or tongue rolling.	G & I
Panting	Noticeably increased respiratory rate (above 36 breaths per minute) with either an open or closed mouth. Noted while the calves are undisturbed as being absent or present	G & I
Play behaviours	The number of animals demonstrating locomotor or social play in the forms of running, bucking, kicking, butting and/or mock fighting. Recorded as being either present or absent.	G & I
Respiratory Rate	Manually count the number of breaths over a 20 second period while the animals are undisturbed.	I
Shivering	Slow and irregular vibration of the body or parts of the body. Noted in undisturbed calves as being present or absent.	G & I
Vocalisation	Utterance recorded as being present or absent. If present in individual calves the frequency and duration were recorded.	G & I
Skin Tent test	Time taken for a pinch on skin taken on the calf's neck to return to the normal position. Noted as a time and then categorised as to whether calves are considered to be dehydrated or not.	I

[1] G represents group level observations while I represents individual level observations

Table 3.3: Injury score categories as defined by Jørgensen and colleagues (2009).

Score	Description
0	No visual wounds/injuries
1	Hair loss
2	Moderate swelling and/or superficial wound
3	Minor cut through skin or obvious swelling
4	Wound through skin with deeper damage
5	Injury resulting in loss of function

3.4 Results

One hundred and two (102) pen level observations and 504 individual calf observations were made, at the 12 meat processing plants across eight regions of the North and South Island of New Zealand. Across the 102 pens a total of 5,910 calves were observed. The median number of calves per pen was 53 (Minimum = 9; Maximum = 172) and the stocking density ranged from 0.21m^2 per animal to 2.72m^2 per animal with a median of 0.44 m^2.

3.4.1 *Group level observations*

Descriptive statistics for percentage of calves in pen engaged a particular behaviour or observed to have a particular sign of poor health from outside the pen are provided in Table 3.4. Lying had the greatest range where 0% - 100% of calves in a single pen were observed to be lying down with a median of 62%. Other more prevalent indicators included faecal soiling (range of 1% - 48%), oral behaviours (0% - 47%), vocalising (0%-27%) and injuries (0%-23%). Other indicators that were less prevalent (<20%) include shivering, ocular discharge, head shaking, nasal discharge and coughing. Panting, huddling & head tilting were not observed in any calves.

The systematic walk-through was completed in all but one of the pens (n=101) because all of the calves in that pen had been standing before observations commenced. Descriptive statistics for percentage of calves standing before, during and after the walk-through are shown in Table 3.5. There was variation in the number of calves standing before the researcher entered the pen (range 0%-95%) and little changed when the researcher entered the pen. There was an increased prevalence of calves standing while the observer was in the pen with a range of 0% - 100% of calves standing (either completing the systematic walk through or when approached).

3.4.2 *Individual level observations*

Sex and breed were recorded for 425 of the 504 randomly selected individual calves. The percentage of females and males was 31.5% (134) and 68.5% (291), respectively. Most calves were considered to be Jersey cross, Kiwi cross or Friesian (Table 3.6).

Time in lairage before the start of the observation period was recorded for 447 of the 504 calves. The maximum time in lairage was 995 minutes (16.5 hours), the minimum was 60 minutes, and the median was 100 minutes (Figure 3.1).

Figure 3.2 shows the frequency distribution of skin tent test times. The percentage of calves classified as moderately dehydrated calves was 63.1% when moderate dehydration was defined as the skin taking two or more seconds to return to normal after tenting (after: Constable et al., 1998). This figure dropped to 24.8% when moderate dehydration was defined as the skin taking three or more seconds to return to normal after tenting (after: Walker *et al.,* 1998). Ten calves (2%) had skin tent times of 5 seconds or longer.

Of the 504 calves observed, 110 (21.8%) had decreased respiration rate, 275 (54.6%) exhibited normal respiration and 119 (23.6%) had increased respiration rate (Figure 3.3).

Table 3.7 shows the prevalence of behavioural and health indicators in the 504 individual calves assessed. The most prevalent indicators (observed in >23% of calves) were dehydration, faecal soiling, nasal discharge, lying, oral behaviours, increased respiratory rate and ocular discharge. For the 221 calves that had some degree of faecal soiling, 130, or 59%, were considered moderately dehydrated when using the two second cut-off for the skin tent test and 56, or 25%, were classified as moderately dehydrated when the skin tent was three seconds or higher. Of the 504 calves, 205 (40.7%) had some degree of serous nasal discharge. One hundred and sixteen (23%) calves were observed with some degree of ocular discharge; 2 of which had a mucopulurent type secretion and the remaining 114 had a serous secretion. Of the 193 calves (38.3%) recorded as lying, 3 were lying in lateral recumbency and the rest in sternal recumbency. The three calves that were observed in lateral recumbency remained in that position for the entire observation period.

For the 139 calves (27.6%) observed demonstrating an oral behaviour 8 were orally manipulating an object and cross-sucking, 122 were only orally manipulating an object and 25 were cross-sucking. Only 22 calves (4.4%) presented with some degree of injury including hair loss (8 calves), moderate swelling and/or a superficial wound (8 calves), minor cut through the skin or obvious swelling (4 calves), or wound through the skin with deeper tissue damage (2 calves). Six (1.2%) calves vocalised during the two minute observation period. Of these six calves, three vocalised once, one calf vocalised twice, one calf vocalised three times and one calf four times.

3.4.3 *Statistical analysis of impact of time in lairage and time in season*

The most prevalent indicators were: dehydration, at both the two second and the three second cut off; faecal soiling; nasal discharge; lying; oral behaviours; increased respiratory rate; and ocular discharge. Table 3.8 describes the univariable association between each of the indicators and time in lairage and time in season. Graphical representations of the seven prevalent potential welfare indicators versus time in lairage and week in study are presented below (Figures 3.4-3.7; Appendix B). Results from the separate mixed effects logistic regression models exploring the impact week of study (week) or time in lairage (minutes) on indicators of health and behaviour are presented in Table 3.9.

Table 3.4: Descriptive statistics for percentage of calves in a pen that demonstrated specific behaviours when observed from outside the pen. Data from a cross-sectional survey of 12 meat processing plants and 102 pens during the 2016 bobby calf season. Recorded with no decimal places.

Variable	Minimum	25th Percentile	Median	75th Percentile	Maximum
Number of Calves	9	34	53	79	172
Lying (%)	0	36	62	78	100
Faecal Soiling (%)	1	10	16	24	48
Oral Behaviours (%)	0	7	12	18	47
Vocalising (%)	0	2	5	8	27
Injury (%)	0	0	0	2	23
Shivering (%)	0	0	0	1	17
Ocular Discharge (%)	0	1	3	4	14
Head Shaking (%)	0	0	0	1	8
Nasal Discharge (%)	0	0	1	3	7
Playing (%)	0	0	0	0	4
Coughing (%)	0	0	0	0	2
Head Tilting (%)	0	0	0	0	0
Huddling (%)	0	0	0	0	0
Panting (%)	0	0	0	0	0

Table 3.5: Descriptive statistics for the systematic walk-through conducted in 101 of the 102 pens. Reported are the percentage of calves in a pen that were observed to be standing before a researcher entered the pen, immediately after they enter the pen, during a systematic walk through of the pen as well as when approached, and five minutes after the walk through. Data are from a cross-sectional survey of 12 meat processing plants during the 2016 bobby calf season.

Stage	Minimum	25^{th} Percentile	Median	75^{th} Percentile	Maximum
Before entry	0	25	37	56	95
Pen entry	0	26	37	56	95
Observer in pen	0	39	54	71	100
5 mins after	0	43	57	74	100

Table 3.6: Breed composition of sample. Data from a cross-sectional survey of 504 calves at 12 meat processing plants during the 2016 bobby calf season.

Breed	Number	%
Jersey Cross/Kiwi Cross	193	38
Friesian	108	21
Beef Cross/Friesian Cross	75	15
Jersey	49	10
Not recorded	79	16
Total	504	100

Table 3.7: Number and percentage of calves exhibiting behaviours and observed to have a potential indicator of poor health present. Data was from a cross-sectional survey of 504 calves at 12 meat processing plants in New Zealand conducted from June to October 2016.

Variable	Number	%
Dehydrated[a]	318	63.1
Faecal Soiling	221	43.8
Nasal Discharge	20	40.7
Lying	193	38.3
Oral Behaviours	139	27.6
Dehydrated[b]	125	24.8
Increased Respiration Rate[c]	119	23.6
Ocular Discharge	116	23
Injury	22	4.37
Head Shaking	13	2.6
Shivering	9	1.8
Vocalising	6	1.2
Coughing	0	0
Head Tilting	0	0
Panting	0	0

[a] A calf was classified as moderately dehydrated if the skin took 2 or more seconds to return to normal after tenting.

[b] A calf was classified as moderately dehydrated if the skin took 3 or more seconds to return to normal after tenting.

[c] Increased respiratory rate was defined as >36 breaths per minute.

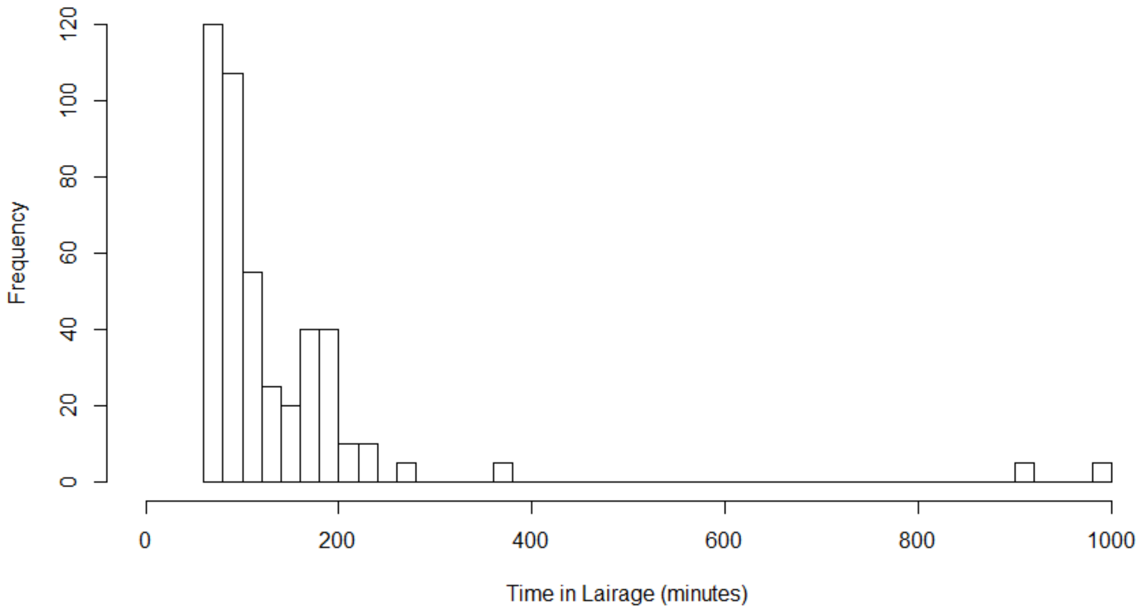


Figure 3.1: Frequency histogram for time in lairage before start of observation for the 102 pens observed during group level observations.

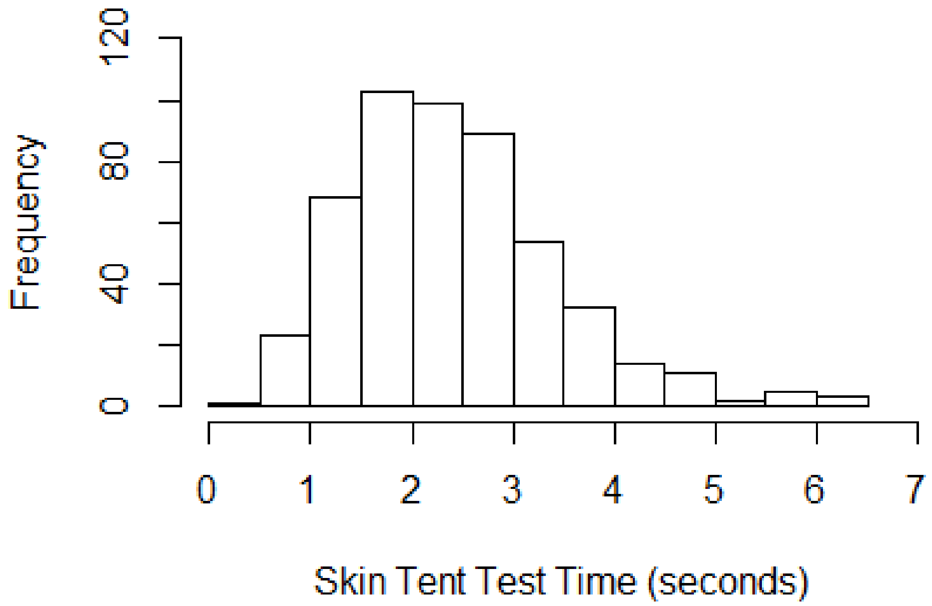


Figure 3.2: Frequency histogram of skin tent test times for 504 individual calves.

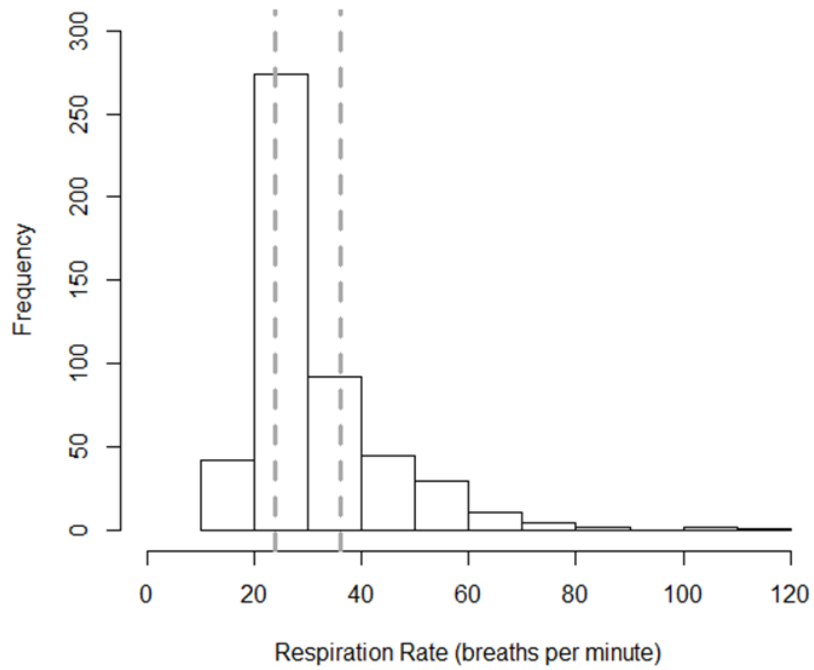


Figure 3.3: Frequency histogram of respiration rates for 504 individual calves. The dashed lines are at 24 and 36 breaths per minute and represent the limits for normal respiration.

Table 3.8: Results from the univariate Kruskal-Wallis tests for the prevalent health and behavioural welfare indicators observed in individual calves for 504 bobby calves recorded during a cross-sectional study conducted in 12 meat processing plants throughout New Zealand during the 2016 bobby calf season.

Indicator	Variable	Kruskal-Wallis p-value
Dehydration (2s)	Week of Study	<0.005
	Time in Lairage	0.002
Dehydration (3s)	Week of Study	0.055
	Time in Lairage	<0.005
Faecal soiling	Week of Study	<0.005
	Time in Lairage	0.610
Nasal Discharge	Week of Study	<0.005
	Time in Lairage	0.005
Ocular Discharge	Week of Study	0.854
	Time in Lairage	0.998
Lying	Week of Study	0.664
	Time in Lairage	0.112
Oral Behaviours	Week of Study	0.054
	Time in Lairage	0.080

Table 3.9: Results from the separate mixed effects logistic regression models exploring the impact week of study (week) or time in lairage (minutes) on indicators of health and behaviour. Data from 504 bobby calves observed during a cross-sectional study conducted in 12 meat processing plants throughout New Zealand during the 2016 bobby calf.

Indicator	Variable	Beta	SE	OR(95%CI)	p-value
Respiratory Rate	Week of study	0	0.2	1.04 (0.71-1.53)	0.84
	Time in lairage	**0**	**0**	**0.10 (0.97-0.99)**	**0.00**
Ocular Discharge	Week of study	0	0	0.97 (0.92 - 1.03)	0.34
	Time in lairage	0	0	1.00 (1.00-1.00)	0.19
Nasal Discharge	Week of study	**0.3**	**0.1**	**1.31 (1.18-1.46)**	**0.00**
	Time in lairage	0	0	1.00 (1.00-1.00)	0.37
Faecal Soiling	Week of study	**1.1**	**1.1**	**1.11(1.06-1.17)**	**0.00**
	Time in lairage	1	1	1.00(1.00-1.00)	0.43
Dehydration (2 seconds)	Week of study	**1.1**	**1**	**1.09 (1.03-1.15)**	**0.00**
	Time in lairage	0	1	1.00 (1.00-1.00)	0.42
Dehydration (3 seconds)	Week of study	1	1	1.00 (0.95-1.06)	0.92
	Time in lairage	0	1	1.00 (1.00-1.00)	0.06
Oral Behaviours	Week of study	1	0.9	0.96 (0.90-1.03)	0.22
	Time in lairage	0	1	1.00 (1.00-1.00)	0.07
Lying	Week of study	1.1	1	1.07 (1.00-1.15)	0.06
	Time in lairage	**0**	**1**	**1.00 (1.00-1.01)**	**0.00**

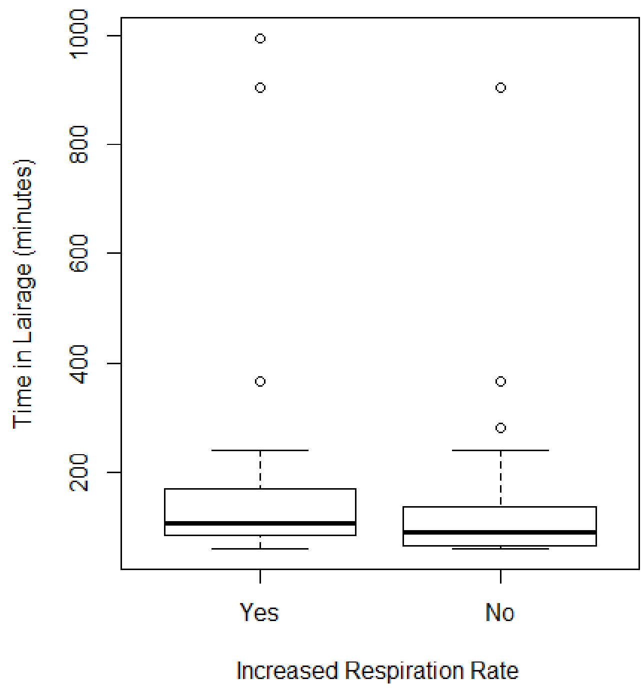


Figure 3.4: Box plot showing the relationship between increased respiratory rate and time in lairage (minutes). Increased respiratory rate is categorised as either no (normal or decreased respiratory rate) or yes (increased respiratory rate).

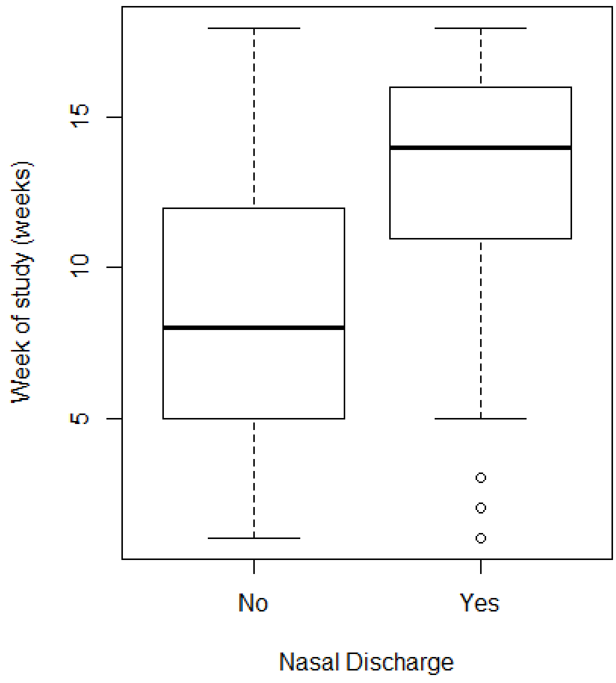


Figure 3.5: Box plot showing the relationship between nasal discharge and week of study (weeks). Nasal discharge is categorised as either no (absent) or yes (present).

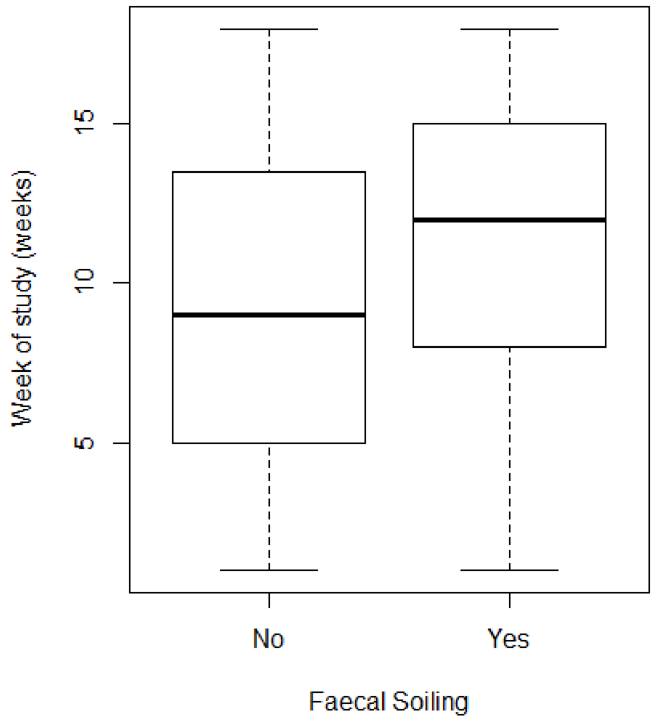


Figure 3.6: Box plot showing the relationship between faecal soiling and week of study (weeks). Faecal soiling is categorised as either no (absent) or yes (present).

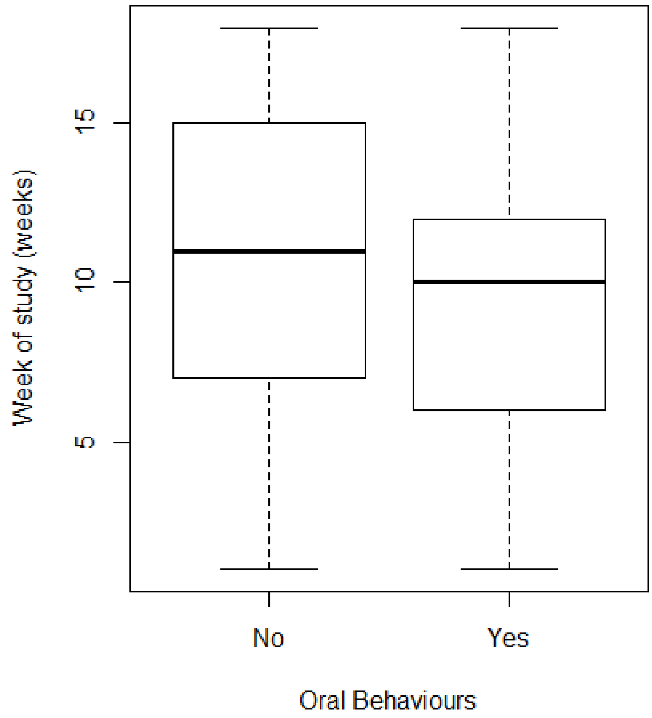


Figure 3.7: Box plot showing the relationship between oral behaviours and week of study (weeks). Oral behaviours are categorised as either no (absent) or yes (present).

3.5 Discussion

The objective of this study was to determine the prevalence of potential indicators of poor welfare in New Zealand bobby calves in lairage and to evaluate the effects of time in lairage and week of study on the prevalence of potential welfare indicators. A number of health or physiological indicators were found to be prevalent among calves when assessed individually. Those with prevalence of around 20% or higher were: dehydration, faecal soiling, nasal and ocular discharge, increased respiratory rate and decreased respiratory rate. Faecal soiling was also common when calves were assessed from outside the pen at group level. All are likely to indicate compromised bobby calf welfare. Prevalent calf behaviours in lairage were lying and oral behaviours; the value of these behaviours for indicating poor welfare status in this context is not clear.

3.5.1 *Prevalent health or physiological indicators*

Moderate dehydration, identified in individuals using a skin tent test, was prevalent among calves held in lairage at the processing plants visited. Moderate to severe dehydration is often associated with lethargy, weakness and recumbency (Walker et al., 1998) which likely reflect unpleasant experiences for the animal and thus compromised welfare as defined by Mellor and Beausoleil (2015). When dehydration is the result of diarrhoea, as was suggested by the concurrent high prevalence of faecal soiling, those animals would likely experience additional negative experiences such as sickness, abdominal discomfort and perianal pain.

When using the skin fold test, our study reported high levels of dehydration in calves in lairage. Previous work assessing bobby calves in lairage facilities in New Zealand found that the majority of spring calves assessed at a single New Zealand meat processing plant (6836 of 7169 assessed) were of an ‘acceptable’ state upon unloading (Stafford *et al.,* 2001). ‘Acceptable’ reflected calves that were strong, able to walk unassisted, bright and alert, round sided and had a dry umbilicus. In contrast, 'unacceptable' calves were unable to stand or walk, were seriously injured or were extremely weak, while 'marginal' calves were hollow sided, had wet umbilicus, had an immature appearance, were weak or slow on their feet. The biochemical profiles of the acceptable and marginal calves did not suggest they were dehydrated. However, the unacceptable calves had high packed cell volumes and plasma urea concentrations which are indicative of dehydration (Stafford *et al*., 2001).

High levels of dehydration are not supported by Todd and colleagues (2000) concluded that none of the fifty six 5-10day old New Zealand calves sampled after transportation were dehydrated. They examined the effects of fasting for 30 hours and 12 hours transportation on packed cell volume and total plasma protein concentrations in calves. This is supported by the findings of Grigor *et al.* (2001) who analysed concentrations of plasma albumin, sodium and the plasma osmolality in ninety six 10 day old male calves that were transported for 1 hour and held in lairage for up to 12 hours in the United Kingdom. They found no effect of lairage duration on the clinical or biochemical signs of dehydration (Grigor *et al.,* 2001). Kent & Ewbank (1986a) also found no evidence of dehydration in 1-3 week old calves that were fasted during transport in the United Kingdom. They studied changes in haematocrit and serum protein concentrations in eighteen calves that had been fasted for 18 hours (six calves) and others that had been transported and fasted for 6 hours (six calves) and 18 hours (six calves). In contrast, Warris *et al.* (1995) and Knowles *et al.* (1997) detected dehydration in young calves after transportation in the United Kingdom. Warris and colleagues (1995) assessed twenty four 12-18 month old cattle that were transported between 5 and 15 hours. Increases in albumin, osmolality and total plasma protein indicate slight dehydration while the animals were fasted and this was quickly corrected when they were provided water. Knowles and colleagues (1997) found an increase in plasma total protein, albumin, and osmolality and packed cell volume with increased journey length in 112 less than one month old calves that were transported for 24 hours. The variation between the studies could reflect differences attributed to geographical location and the differing ages of the animals. It is also possible that the differences are due to the fact dehydration was assessed differently in each of these studies.

The cause of dehydration in the present study was not determined. There are two plausible hypotheses. Firstly is that dehydration could be the result of diarrhoea. The second is that it was due to when they last had access to feed or the opportunity to drink water. The observed prevalence of dehydration is consistent with the high prevalence of faecal soiling observed on individual calves and in groups, suggesting that dehydration may have been associated, at least in part, with scouring. Calf diarrhoea is attributed to both infectious and nutritional factors (Foster & Smith, 2009; Cho & Yoon, 2014). Nutritional diarrhoea results from a change in feed type, composition or volume and is less common in young calves than infectious diarrhoea which is attributed to pathogen exposure (Vermunt, 1994; Constable, 2009; Foster & Smith, 2009; Cho & Yoon, 2014). Both aetiologies can result in severe dehydration (Walker *et al.,* 1998).

It is also possible that dehydration was the result of prolonged food/water deprivation. To illustrate, Werner-Omazic *et al.* (2013) found depriving healthy calves, aged up to 15 days, of food and water for 24 hours resulted in mild dehydration (6% lost body weight). Similarly, duration of food deprivation during transport influenced the degree of dehydration in older cattle; mild dehydration (5%) occurred after a 5 hour journey and mild-moderate dehydration (6.5 – 7%) after 10 and 15 hours of travel respectively (Warriss *et al.,* 1995).

In the present study, we were unable to determine how long since calves had left the farm of origin. However, by regulation, bobby calves must be slaughtered within 24 hours of their last feed (MPI, 2017). While some plants provide some bobby calves with milk replacer in lairage, few record details of food provision. Even if they had, there was no way of knowing the timing and volume ingested by individual calves. Likewise, although water was made available to the calves, whether, when and how much they drank was unknown. Thus, the cause of dehydration could not be ascertained. Nonetheless, the high prevalence of clinically significantly dehydration in calves in lairage is consistent with condemnation and post-mortem findings (Boulton *et al.,* 2017).

Some caution is required in interpreting the prevalence of dehydration using the skin testing method applied in this study. While the method was based on previous studies of dehydration in neonatal calves, there were some factors that may influence interpretation of the time for skin to return to normal as a reflection of degree of dehydration. First, previous studies on calves used a 'pinch and twist' method for tenting skin (e.g. Constable *et al.,* 1998, Walker *et al.,* 1998) while the skin was only pinched in this study. Second, even in studies using the same method of tenting, the return time reported to reflect 'moderate' dehydration varies. These differences may be due, in part, to the different locations at which tenting was performed in those studies or due to other aspects of measurement or experimental context.

To illustrate, while Walker *et al.* (1998) only classified calves as moderately dehydrated when skin took more than three seconds to return to normal, they performed their test midway along the lateral thorax of calves. In horses, anatomical location of testing influenced the time skin took to return to normal after tenting (Pritchard *et al.,* 2008). Thus, a three second cut-off to indicate moderate dehydration in young calves may not be appropriate when tenting is measured on the back of the neck.

In contrast, Constable *et al.* (1998) tested skin tenting in the same neck location as was used in the current study and classified return times of 2-5 seconds at this location as reflecting moderate dehydration (noted as 8-10%). Thus, this shorter cut-off time may be most appropriate for determining prevalence of clinically significant dehydration in the current study. According to this classification, almost two thirds of the calves assessed in lairage in 2016 were moderately dehydrated. Although the difference in application of the tent (i.e. pinch and twist used previously) might influence time to return, our use of pinch alone would be expected to shorten rather than prolong skin return time, suggesting that, if anything, prevalence was underestimated in the current study.

Ten calves (2%) had skin tent return times of five seconds or longer. According to Smith (2009), calves with neck skin tent times of five seconds or longer in the context of scouring would be severely dehydrated (10-12% body weight) and comatose. In contrast, in an experiment using osmotic (nutritional) rather than infectious diarrhoea to cause dehydration, calves that were severely dehydrated (14% body weight) were still able to stand and suckle at feeding times, though they preferred to lie the rest of the time (Walker et al. 1998). Reporting the behaviour and general demeanour of the calves would provide more information on the clinical significance and welfare implications of skin tent test results. In conclusion, this measure was chosen as an indicator of dehydration as it is practical, cheap and easy to perform. However, concurrent evaluation of the calves' general demeanour and degree of enophthalmos (eyeball recession) should be undertaken in future studies to support the findings of the skin tent test in terms of hydration status (Smith, 2009).

There was no significant relationship between time in lairage before the observational period and dehydration using either the 2 or 3 second cut off. This result agrees with the findings of Grigor and colleagues (2001) who reported no effect of the duration in lairage on the occurrence of dehydration. There are two factors that could have influenced this result. Firstly, there is very little variation in time in lairage, where although the maximum time was 995 minutes (16 and a half hours), the minimum time was 60 minutes and the median was 100 minutes (see Figure 3.1). The second point to consider is that the duration of transport and the time since the calf's last feed is unknown. Time in lairage is not indicative of the whole time the calf is fasted. Moreover, calves are provided with a water source during lairage and have the opportunity to rehydrate. In this present study there was a significant relationship between week of study and dehydration (2 second cut off) in the mixed effect model with region as a random effect. This result could be due to: the increased risk of faecal soiling from diarrhoea as the study progressed; the increase in ambient temperature which may result in heat stress in high stocking densities especially towards the later part of the season.

Nearly a quarter of individual calves exhibited increased respiratory rate. This variable was characterized as closed mouth hyperventilation, rather than open-mouthed panting for thermoregulation. While increased respiration rate could be exercise related, none of the calves individually assessed were observed locomotor playing. Thus, this variable likely reflected increased chemical drive to breathe, due to either respiratory or metabolic acidosis (Bleul *et al.*, 2007). Metabolic acidosis refers to a disturbed acid base balance where there is a reduction of bicarbonate ions which reduces the pH and leads to respiratory compensation through increased respiration rate in order to decrease the partial pressure of carbon dioxide (Edwards, 2008). The reduction in pH stimulates central chemoreceptors controlling respiration (Mitchell & Singer, 1965; Constable, 1999; Edwards, 2008).

The absence of coughing makes respiratory infection and impaired gas exchange (i.e. respiratory acidosis, increased partial pressure of carbon dioxide) a less likely cause of increased respiratory rate than metabolic acidosis due to scouring. Metabolic acidosis can occur due to the loss of water, electrolytes and bicarbonate due to severe diarrhoea (Naylor, 1987; Berchtold, 2005). Again, the high prevalence of faecal soiling supports this interpretation.

The risk of increased respiratory rate lessens with progressing time in lairage in the logistic regression model where the random effect of region was accounted for. The transportation process is a novel experience and lairage facilities are a different environment with new stimuli. Activation of the hypothalamic-pituitary-adrenal axis can result in an increased respiration rate (Fike & Spire, 2006). While we would expect calves that had been in lairage for at least an hour to have settled, the arrival of stock trucks, movement of animals and associated noises may have disrupted the calves and resulted in the physiological stress response. Calves were left for at least an hour before observations were made and this 'settling' time may have allowed them to adjust to the new environment and their respiration decrease back to a 'normal' rate. A decrease would also be seen in calves that spend more time in lairage as they have had more time to settle and adapt to their surroundings. Over 20% of the calves had a respiratory rate less than 24 breaths per minute. There is very little evidence suggesting the aetiology of decreased respiratory rate, however, it has been linked with painful conditions as well as severe metabolic alkalosis as a precautionary measure to preserve carbon dioxide (Divers & Peek, 2008).

Finally, ocular and nasal discharges were commonly noted in individual calves. While such signs can be indicative of infection, the secretions were serous in almost all calves. This suggests that ocular and nasal discharge in the calves assessed was due to acute irritation, rather than infection. Irritation may have occurred due to dust or noxious gases present during transport or in lairage. This is supported by the findings from Nordstrom & McQuitty (1975) who demonstrated that concentrations of ammonia (above 0.065ml/l) and hydrogen sulphide (above 0.02ml/l) cause irritation and inflammation of mucosal membranes and calves present with ocular and nasal discharge (Nordstrom & McQuitty, 1975; Randall, 1993). Eye irritation, in particular, could be associated with discomfort for the calves. Serous ocular discharge has also been noted as a clinical sign of disease (McGuirk, 2008). While the aetiology of the discharge cannot be determined from this study, Jorgensen and colleagues (2017) noted a relationship between eye scores (differing severities of ocular discharge) and stocking density on farm. Calves in pens with less space were more likely to have more severe eye scores (Jorgenson *et al.,* 2017). This study found no significant association between ocular discharge and time in lairage or week of study in either model.

In this present study nasal discharge increased with week in the study. This result supports that of Bršćić and colleagues (2012) who found that the prevalence of nasal discharge in veal calves increased over time on the farm. They considered that nasal discharge and coughing are not necessarily signs of severe disease however they identified that increased air circulation was a risk factor for nasal discharge. Further evidence supporting air circulation as a risk factor is reported by Lundborg and colleagues (2005) who found the presence of a draught (measured as wind velocity greater than 0.5 meters per second) increased the risk of increased respiratory sounds in calves.

Lying and oral behaviours were prevalent behaviours observed in bobby calves during the 2016 season. Care must be taken in the interpretation of behaviours in terms of calf welfare status; prevalence alone does not indicate welfare compromise in calves in lairage. The behaviours assessed in this study were chosen based on a review of the literature, suggesting some relevance to calf welfare.

While the expression of oral behaviours was prevalent at both group and individual level, the meaning of these behaviours for calf welfare in the lairage context is unclear. Manipulation of objects in the pen and sucking on other calves were the most commonly observed oral behaviours; these could indicate hunger, boredom or frustration, all of which are negative experiences which impact detrimentally on welfare if intense (Mellor and Beausoleil, 2015). It is plausible that the calves observed were hungry; however, as we were unable to determine how long since they left the farm (i.e. access to food), this is only speculative. In this study the prevalence of oral behaviours was observed to decrease over the weeks of the study. There is no evidence from the literature to support this observation, however, it could be due to more frequent slaughtering during the middle of the season so calves are held in lairage for a shorter time and are not transported as far. Previous works have concluded that competitive behaviours and crowding at the feeder are indicative of hunger (Jensen & Holm, 2003; von Keyserlingk *et al.*, 2004; Vieira *et al.,* 2008; Battini, *et al*., 2014), however, these are irrelevant to assessments made in lairage where no feed is presented. Alternatively, the oral behaviours observed could be forms of play in calves of this age, although there is no information to support this interpretation currently.

Lying was highly prevalent both in undisturbed groups of calves and when calves were assessed individually. Lying itself may not be a useful indicator of welfare status as calves will lie in lairage for various reasons. However, a preference to lie has been associated with severe dehydration in neonatal calves (Walker et al., 1998), and the postural responses of calves to disturbance may be more indicative of their welfare status. Approximately two thirds of the calves were lying down when undisturbed in lairage and most appeared disinclined to stand when the researcher was in the pen, even when approached directly. Almost no calves stood when the researcher entered the pen, fewer than 10% of those lying stood up when the researcher walked through the pen and only another 10% stood when approached directly towards the head to a distance of 0.5m. This suggests either that calves were lethargic or that they did not perceive the researcher as a threat or a source of food. It would be interesting to know whether the 40% of calves that never stood during the group observation period included those exhibiting signs of diarrhoea and/or dehydration. In any case, lying alone is probably not a reliable indicator of calf welfare in lairage.

3.5.2 *Time in Lairage and Week of Study*

Time in lairage has been considered to be an influential factor associated with calf mortality along with increased transport time (O'Grady & Thomas, 2013). During the start and the end of the processing season, the schedules for each meat processing plant can vary considerably resulting in calves being transported for long journeys to get to an operating plant. This could explain the increase in prevalence of nasal discharge and moderate dehydration at later weeks in the study. Calf mortality tends to increase towards the end of the season (O'Grady & Thomas, 2013) which could be due to an accumulation of pathogens in the calf sheds, or the differing plant processing schedule which accommodates for lower throughput of bobby calves by either reducing the days operating, or closing and referring calves to another plant (O'Grady & Thomas, 2013). Furthermore, O'Grady & Thomas (2013) note that over half of the condemnations or pre-slaughter mortalities in the 2012 bobby calf season were linked to infective diseases. Such diseases commonly result in dehydration, acidosis and toxaemia (O'Grady & Thomas, 2013). If unrecognised during ante-mortem inspections, longer lairage periods can increase the frequency of moribund or dead calves (O'Grady & Thomas, 2013).

Time in lairage before observations only provides a fraction of the information of the animal's history. Transport time was not traced for the calves in this study so the researchers had no idea how long they had been on the livestock truck for or how long since their last feed. The range in lairage times was large (1 hour – over 16 hours). For this reason, regulations are not based solely on time in lairage, but also time off feed.

3.5.3 *Methodological considerations*

It is important to note that at the time of assessment, many of those calves that had arrived at the plant with the poorest welfare status would already have been condemned (on arrival) and excluded from the observations. This is because observations were not undertaken until calves had been in lairage for at least one hour after arrival, and data provided by the plants indicated that about half of calf mortality was due to condemnation on arrival. Thus the observations made in lairage do not reflect the complete picture of calf welfare compromise across the supply chain, as calves identified with at least one of the potential indicators very possibly experienced poor welfare during transport to the plant.

Although a welfare indicator may have been considered as being prevalent, assessment protocols such as the one used in this observational study only provide a snap-shot of welfare state at the time of the observations. Most of the potential indicators reflect the welfare state of the animals over a longer period of time than the observational period (Whay *et al.,* 2003).

Likewise, the level at which observations are made can influence the reported prevalence of potential welfare indicators. For example, the prevalence of faecal soiling and expression of oral behaviour at group level (i.e. from outside the pen) were much lower than when assessed on individual calves. Nasal and ocular discharges were rarely noted in groups of calves but were commonly observed in individuals. At group level it was difficult to accurately identify health indicators and assess behaviours from outside the pen and over such a short period. Thus, these discrepancies are probably due to the inability to accurately identify these indicators from outside the pens. They may also reflect differences in the degree to which the indicator was present. For example, any degree of faecal soiling was scored as present for individual calves, but probably only moderate or severe soiling would be visible from outside the pen. Thus, for accurate assessment of indicators of calf health and welfare in lairage, it appears necessary to enter the pen and assess individual calves, but to also make an assessment of the severity and clinical and welfare significance of the sign.

Panting, huddling and head tilting were not observed during group level observations. While some calves had increased respiratory rate, none were observed technically panting with open mouth respiration. Without measuring the respiration rate for every calf in the pen, subjectively selecting calves with an 'increased respiratory rate' may not have been accurate. Huddling was not recorded due to the stocking densities of the pens. It was impossible to differentiate between overcrowding and huddling for thermoregulatory purposes. Furthermore, it was impossible to accurately assess head tilting for all calves in each pen. Head tilting has been associated with disease in calves (Wilson *et al.,* 2007); however, assessment would have been subjective with potential for human error.

The results presented in this study determine the prevalence of potential indicators of health and welfare in bobby calves in lairage during the 2016 bobby calf season. Results from future studies, while comparable, may vary due to seasonal variation and changes to the regulatory environment.

3.6 Conclusions

From this observational study, dehydration, faecal soiling, increased respiratory rate and ocular/nasal discharge appear to be highly prevalent in bobby calves in lairage and may be useful indicators of aspects of calf welfare. It seems likely that dehydration and faecal soiling reflect diarrhoea, although whether this is nutritional or infectious is unknown. Either way, they are indicative of compromised calf welfare in lairage. The progressive development of these indicators from farm collection through to the point of slaughter should be investigated to better understand the welfare status of calves across the supply chain.

Oral behaviours and lying were prevalent behaviours in lairage and may reflect hunger and lethargy/weakness. However, the links between concurrent physiological or disease status and the expression of these behaviours needs to be validated before their use as indicators of calf welfare in this context.

Chapter 4: General Discussion

The research presented in this thesis has identified a number of potential indicators of bobby calf welfare and analysed the observed prevalence of a sub-set of these indicators in lairage facilities at commercial meat processing plants across New Zealand. A number of areas of potential welfare compromise have been identified in calves in lairage. The findings are important for the bobby calf industry in order to facilitate further reductions in mortality rate and improve the welfare of calves that currently survive to the point of slaughter.

In Chapter 2 the published literature relating to potential indicators of animal welfare was systematically mapped, with the aim of identifying indicators useful for assessing bobby calf welfare. The systematic mapping identified a wide range of potential welfare indicators; 99 different indicators were identified from the 253 articles retrieved. The indicators identified reflected aspects of nutrition, environment, health and behaviour, and many are applicable at either a group or individual level. The indicators identified during this exercise are simply those that have been used in previous attempts to characterise welfare in different situations. No attempt was made to consider the validity, practicality, feasibility or reliability of each indicator. However, demonstrating the validity and practicality of indicators is important if useful conclusions are to be drawn about calf welfare.

The observational study described in Chapter 3 provides insight into the prevalent potential indicators of bobby calf welfare at the meat processing plant. Bobby calves were assessed in consignments (group level) and a random subset of these was also assessed individually. The first objective was to determine the prevalence of potential welfare indicators of poor calf welfare and health in lairage prior to slaughter in the 2016 season. To summarise, dehydration, faecal soiling, increased respiratory rate, ocular discharge, nasal discharge, oral behaviours and lying were moderately prevalent in bobby calves in lairage and these indicators may provide insights into aspects of calf welfare in the supply chain. The prevalence of dehydration and faecal soiling likely due to diarrhoea, however, whether the scouring is of nutritional or infectious in origin is unknown. Scouring and loss of water can lead to metabolic acidosis which can increase respiration rate. The prevalence of nasal and ocular discharges was likely due to irritants, such as noxious gases, or to exposure to drafts. Because the majority of both were serous, severe disease is a less likely cause. Behaviours such as lying and oral behaviours may reflect lethargy and hunger respectively, however, there is weak evidence to support the animals drive to perform such behaviours and these behaviours could be indicative of other experiences. For example, lying may be due to weakness, but it also may be because the calf is resting which is important to young animals. Further investigation is required to validate these indicators and to demonstrate the practicality, repeatability and reliability of measurement during routine processing of calves.

The second objective of Chapter 3 was to evaluate the effect of week in season and time in lairage on the percentage of calves demonstrating the selected prevalent indicators at the individual animal level. The logistic regression model which assessed the relationship between prevalent indicators and the week of study as well as time in lairage after accounting for any effect of region observed that nasal discharge, faecal soiling and dehydration (2 second cut off) were significantly associated with week of the study/season; the prevalence of each indicator increased later in the season. Prevalence of increased respiration rate was inversely proportional to time in lairage, and the prevalence of lying down increased with time in lairage. The decrease in prevalence of increased respiration rate with increasing time in lairage could be explained by physiological stress response. The transportation process is a novel experience which may induce a physiological stress response and thus an increased in respiratory rate. The longer the calves had in lairage the longer they would have had to 'settle' and their respiration rate return to a more normal value. The inversely proportional relationship seen in oral behaviours and increased time in lairage could

The work undertaken in this thesis indicates the prevalence of potential indicators of calf welfare in lairage. In particular, the prevalence of indicators that likely reflect scouring and subsequent dehydration, oral and nasal mucosal irritation and hunger.

4.1 Future Research

Future research should address the validity, reliability, repeatability and practicality of measurement for the potential welfare indicators found to be prevalent in bobby calves in lairage.

Repeatability refers to the ability for an animal to be observed and the same result produced across numerous observations and/or observers (Knierim & Winckler, 2009; Llonch *et al.,* 2015). In the context of bobby calves in lairage, a suitable welfare indicator would need to be able to be successfully assessed by yards men and the resident vet. Reliability refers to being able to produce consistent results when observing numerous animals in the same circumstances (Knierim & Winckler, 2009; Llonch *et al.,* 2015). Feasible indicators are those that can be practically assessed in terms of time, cost and easily given the scenario. In lairage at the meat processing plant, where stocking density is high and a large number of calves can be held in a single pen, a feasible welfare indicator would allow for a fast and affordable assessment of an animal, not necessarily in close range (for example, standing outside a pen). Applying these criteria to potential welfare indicators addressed in the scientific literature relating to bobby calves allows the validity, repeatability, reliability and feasibility of each potential welfare indicator to be considered. Because this study found only a selection of potential indicators to be moderately prevalent, their use in regular assessment of bobby calves in lairage facilities in New Zealand is limited.

Further work should also address welfare of calves across the supply chain. Although the occurrence of the potential indicators recorded in this study provides information regarding welfare of calves in lairage, knowledge of the aetiology and progression of the health or behavioural indicators could allow for welfare compromise to be reduced. This could be achieved by following a sub-set of calves from birth through to slaughter whilst comparing them to a control group of calves that remain on farm. Monitoring behaviours and indicators of health during each part of the supply chain may identify the origin of the problem and where possible would alleviate the sources of compromised welfare.

Furthermore, communication with veterinarians and yards men working at the meat processing plants has highlighted concerns for calves that arrive at the plant and are lying down and lethargic as demonstrated by being to move without assistance. Often the cause of this lethargy is not determined and calves are condemned with no significant post-mortem findings. A study aimed at determining the cause of lethargy, or risk factors associated with lethargic calves that are recumbent upon arrival could provide useful information. This could be achieved by following groups of calves from their farm of origin to the meat processing plant and assessing the conditions on the livestock trucks at different stocking configurations. Such measurements could include: the presence of noxious gases, humidity, temperature, wind velocity, ocular and nasal discharge, and behaviours. Behaviours could be assessed though video recordings as well as transponders attached to calves to monitor time lying during transport. Calves on the lower deck of the livestock truck are closer to the effluent tray and those in the centre of the truck will have restricted ventilation which could lead to a build-up of noxious gases. Furthermore, the trucks used for transporting bobby calves are also used for sheep and the height of each ‘level’ can be restricted. This could influence ventilation as well as comfort for the calves while travelling. Reducing the number of levels of calves per truck could allow for increased ventilation, if more over head space is able to be provided, and would reduce the height in which calves have to be lifted onto the upper level(s) of the truck when being loaded.

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Appendices

Appendix A

This appendix presents the recording forms for group level (A1) and individual level (A2) field observations.

Table A1: Recording form for group level observations used during field observations made at 12 meat processing plants across New Zealand during the 2016 bobby calf season.

Group Level Observations

Pen Number	/		
Time of Arrival		**Time of observations**	
Pen location			
Dimension of pens	Length	x Width	
Shelter provided	Roof	Walls	
Temperature			
Wind speed			
Number of animals in pen			
Number of animals lying in the pen		**Standing**	

Behavioural Observations

	Number of animals per group	% of group
Shivering		
Panting		
Huddling		
Oral Behaviours		
Vocalisation		
Head tilting		
Head shaking		

Behaviours demonstrating

Health Observations

	Number of animals per group	% of group
Coughing		
Hampered respiaration		
Severe Ocular discharge		
Severe Nasal discharge		

Faecal Soiling

	Number of animals per group	% of group
No		
Moderatly Dirty		
Extremely Dirty		

Injury	# of animals	% of group	
0			No visual wounds/injuries
1			Hairloss
2			Moderate swelling and/or superficial wound
3			Minor cut through skin or obvious swelling
4			Wound through skin with deeper damage
5			Injury resulting in loss of function

Walk-through completed Y / N (only if there are calves lying down)

Numer of calves lying immediatedly prior to walk-through

Number of calves that got up when observer first entered the pen

Number of calves that get up during systematic walk

Number of calves that get up when approached within 0.5m (after walk-through)

Number of calves that did not get up (non-ambulatory)

Exhaustion?

Number of calves that return to lying 5minutes after walk-through

Comment on position lying

Comment on position lying

Water source working Y / N

Comments and Notes
Diarrhoea

Table A2: Recording form for individual level observations used during field observations made at 12 meat processing plants across New Zealand during the 2016 bobby calf season.

<u>Individual Level Observations</u>

Pen Number	/	Animal Number	/
Calf standing	Y / N	If no, lying position	

Indicators

Shivering

Yes	
No	

Panting

Normal respiration		20-40 breaths per minute
Mild Heat Stress		Closed mouth breathing w/ respiration elevated over 20-40 breaths/min
Panting		Open mouth breathing with respiration elevated over 40 breaths/min

Respiration Rate		breaths per minute
Hampered respiation		
Coughing		

Vocalisation

Vocalising?		Y / N
If yes	Rate over 2 minutes	
	Duration	

Oral Behaviours Y / N

Manipulating an object		biting, suckling, licking
Manipulating a calf		cross-suckling
Tongue Playing		
Other		

Head tilting	
Head shaking	

Faecal Soiling

No	
Moderatly Dirty	
Extremely Dirty	

Injury

0		No visual wounds/injuries
1		Hairloss
2		Moderate swelling and/or superficial wound where skin not perforated
3		Minor cut through skin or obvious swelling
4		Wound through skin with damage to deeper tissues
5		Injury resulting in loss of function

Ocular discharge present Y / N

If yes: Mild [ ] Severe [ ]

Nasal discharge present Y / N

If yes: Mild [ ] Severe [ ]

Dehydration Time for skin to return to normal after skin tent:

General demeanor	Bright	Tired	Weak	
Navel	Wet	Raw	Dry	
Eyes	Bright	Dull	Closed	
Ears	Forwards	Back	Alert	
Coat	Dry	Clean	Wet	Dirty
Hooves	Strong	Soft/undeveloped		

Sex

Breed

Comments and Notes

Diarrhoea
Lesions
Lameness
Ectoparasitism
Mucous Membranes

Appendix B

This appendix presents the results of the graphical analyses of the relationship between the more prevalent indicators and time in lairage as well as week in study. Both significant (already presented in results of chapter three) and non-significant results are presented.

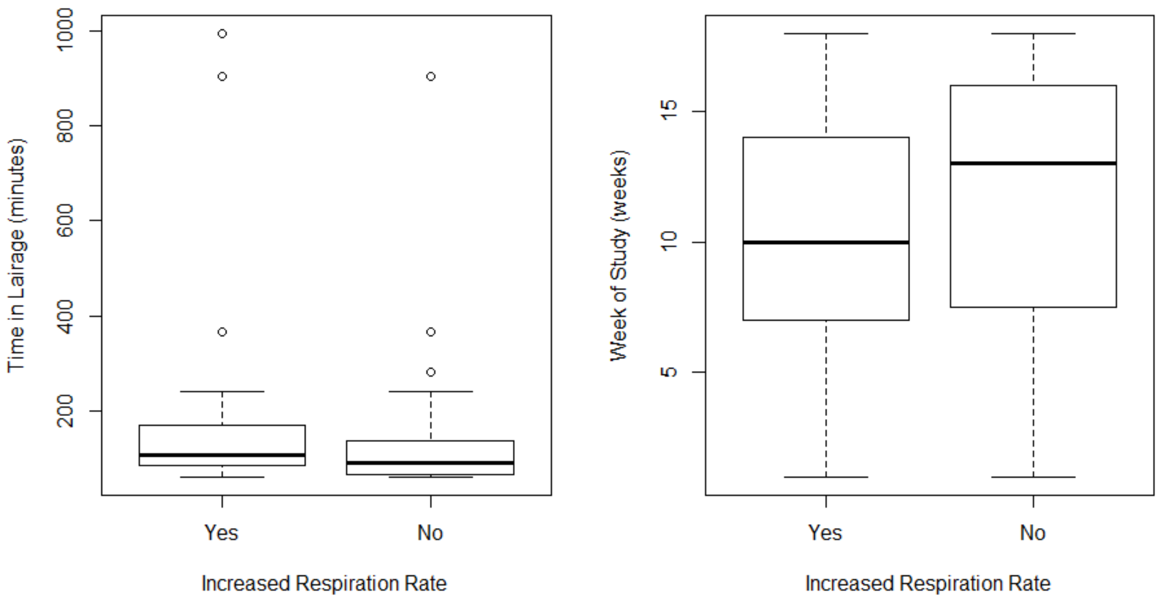

Figure B1: Box plots showing the relationship between respiration and time in lairage (minutes) and between respiration and week of study (weeks). Respiration is categorised as either decreased (<24breaths per minute), normal (24-36 breaths per minutes) or increased (>36 breaths per minute).

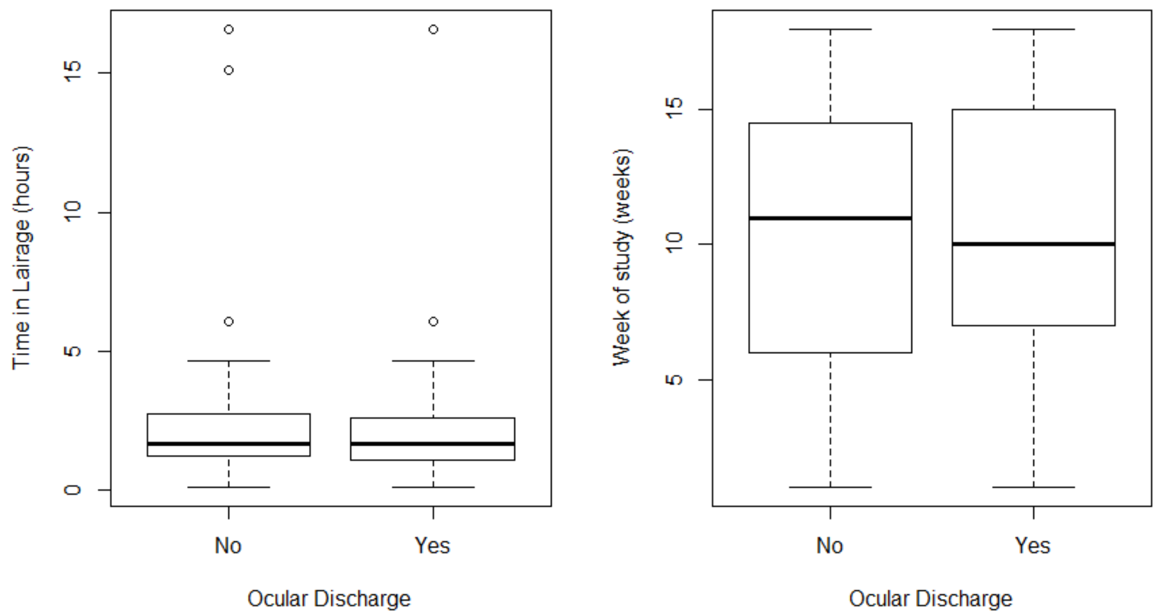

Figure B2: Box plots showing the relationship between ocular discharge and time in lairage (minutes) and between ocular discharge and week of study (weeks). Ocular discharge is categorised as either no (absent) or yes (present).

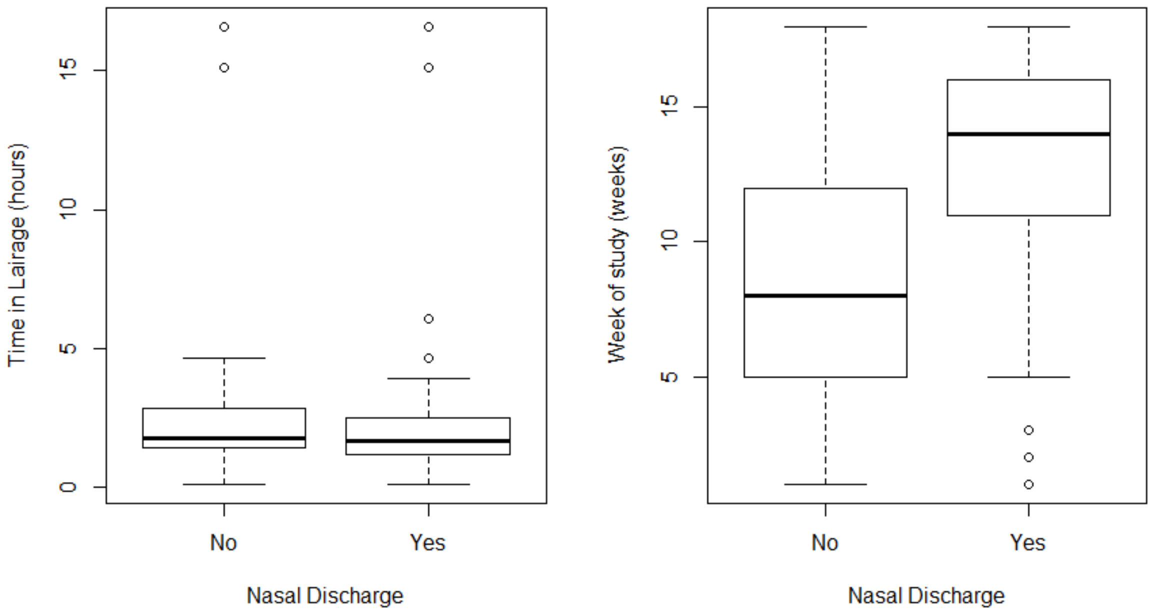

Figure B3: Box plots showing the relationship between nasal discharge and time in lairage (minutes) and between nasal discharge and week of study (weeks). Nasal discharge is categorised as either no (absent) or yes (present).

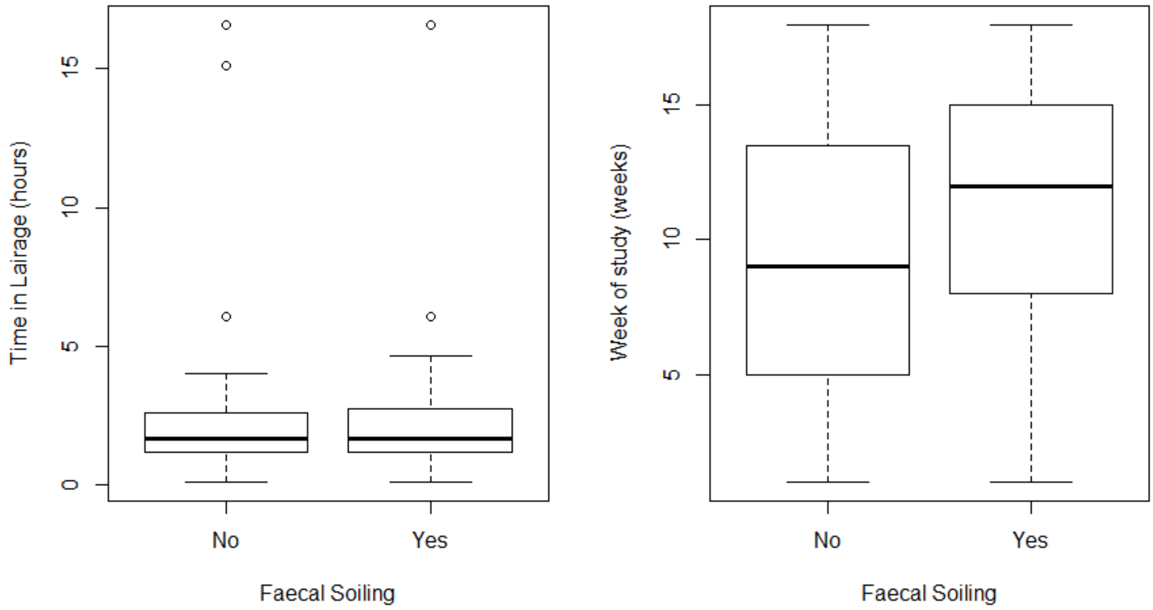

Figure B4: Box plots showing the relationship between faecal soiling and time in lairage (minutes) and between faecal soiling and week of study (weeks). Faecal soiling is categorised as either no (absent) or yes (present).

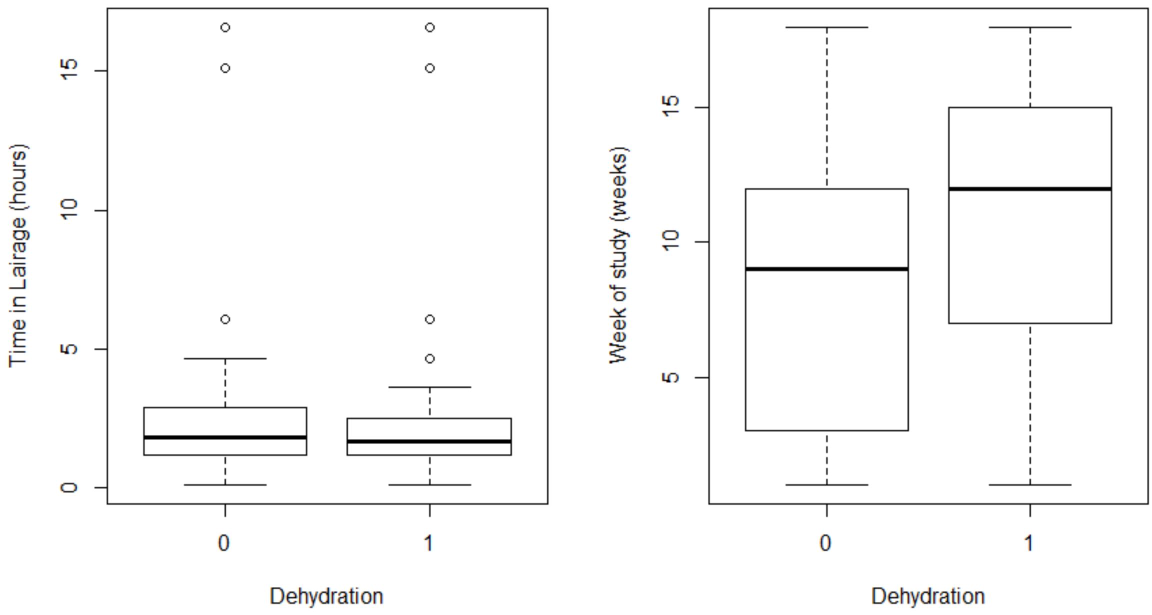

Figure B5: Box plots showing the relationship between dehydration as observed through a skin tent test (considered dehydrated at greater than 2 seconds) and time in lairage (minutes) and between dehydration (2s) and week of study (weeks). Dehydration is categorised as either no (adequately hydrated) or yes (dehydrated).

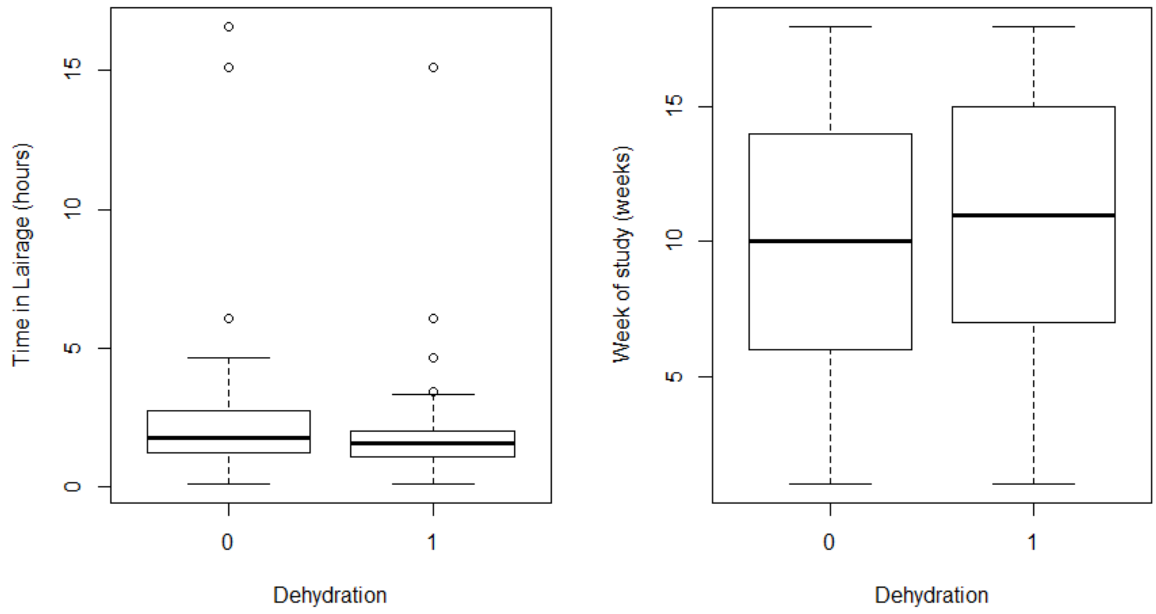

Figure B6: Box plots showing the relationship between dehydration as observed through a skin tent test (considered dehydrated at greater than 3 seconds) and time in lairage (minutes) and between dehydration (3s) and week of study (weeks). Dehydration is categorised as either no (adequately hydrated) or yes (dehydrated).

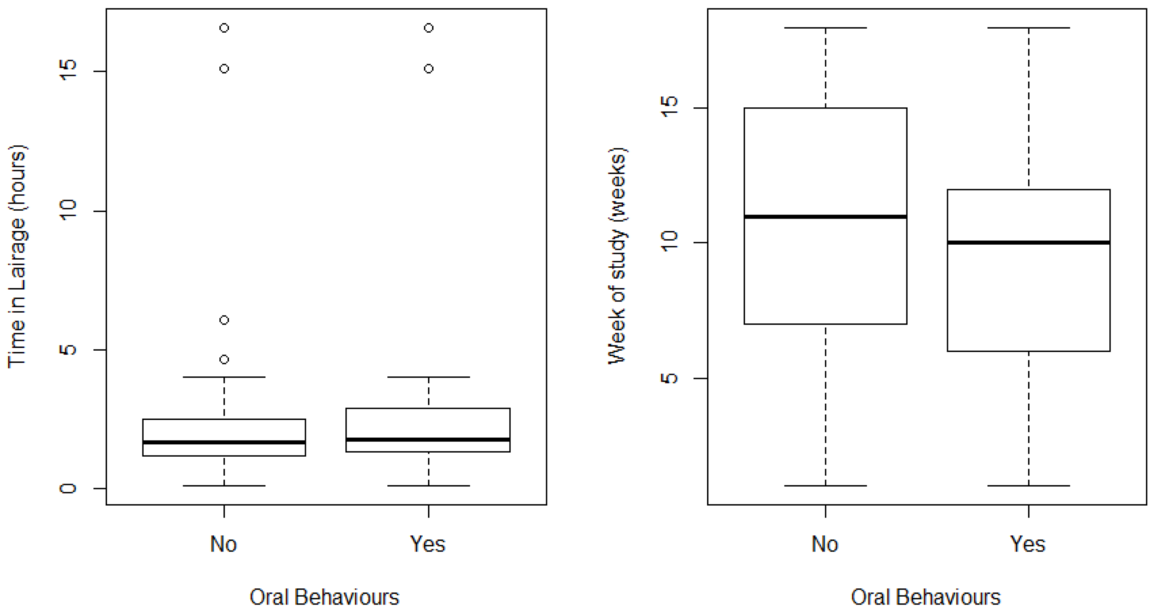

Figure B7: Box plots showing the relationship between oral behaviours and time in lairage (minutes) and between oral behaviours and week of study (weeks). Oral behaviours are categorised as either no (absent) or yes (present).

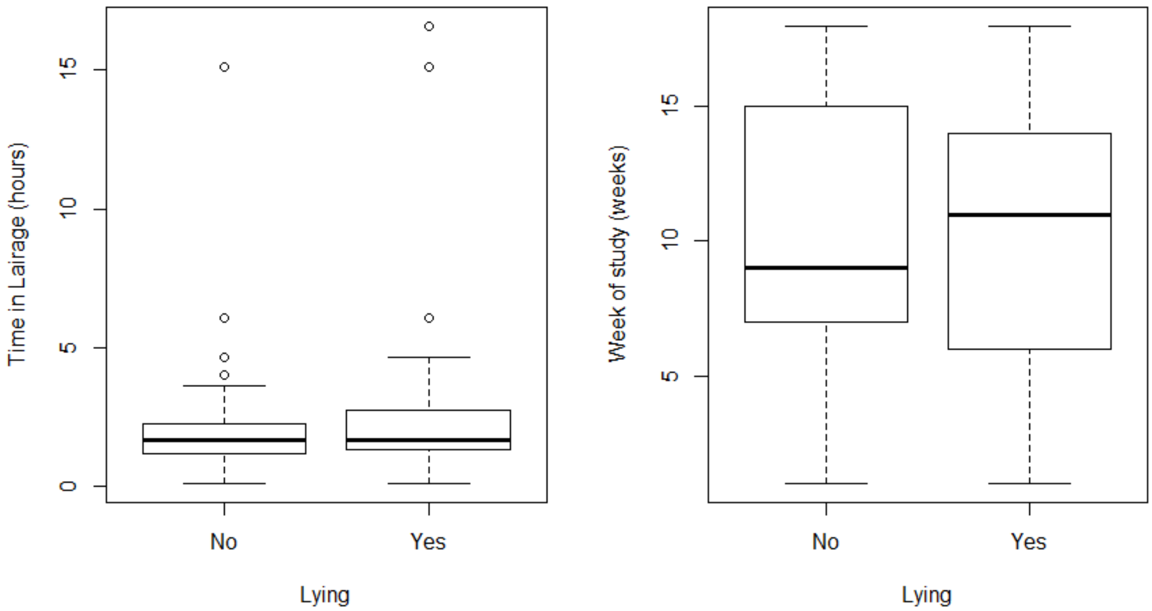

Figure B8: Box plots showing the relationship between calves lying down and time in lairage (minutes) and between calves lying and week of study (weeks). Calves lying down are categorised as either no (absent) or yes (present).