animals

Farm Animal Transport

Edited by
Claire A. Weeks

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Editor

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About the Editor

Claire A. Weeks (Honorary Senior Research Fellow) has researched and reviewed the transport of most species of farmed animals and horses during her career. Her focus has been on the transport of poultry, investigating vehicle design, stocking density, and thermal stress during transit for pullets, layers, and broilers with numerous papers and book chapters. She has worked with NGOs, governments, and industry to ensure that recommendations are evidence-based and has developed a close relationship with industry facilitating the implementation of improved practices during commercial transport. Claire is currently a member of the working group developing a scientific opinion on the welfare of caged farm animals during transport for the European Food Standards Agency (EFSA)'s Animal Health and Welfare Panel reporting to the European Commission.

Preface to ”Farm Animal Transport”

This Special Issue considers the handling and transport of farmed land animals, of which there are some 70 billion produced annually. About two-thirds are farmed intensively within a segmented food chain at some distance from urbanised consumers of animal products. In the opening paper, Temple Grandin, who has spent a lifetime dedicated to improving animal handling, uses her predominantly North American experience to discuss reasons for the persistence of poor production and handling practices that have negative animal welfare and economic consequences. In many parts of the world, consumer awareness is driving the implementation of assurance schemes and legislation that aim to safeguard animal welfare at all stages, including transportation. The second paper explores consumer perceptions of sheep and cattle transport within and from Australia, where incidents in livestock transport by sea to the Middle East often hit the news. A review of the heat load and mortality experienced by cattle and sheep during sea transport in conditions of overcrowding and high ambient temperatures and humidity highlights the need for more research if such consignments are permitted to continue. Encouragingly, evidence emerges from Uruguay of the benefits of welfare training of truck drivers where the use of flags reduced the load time for cattle when compared with the use of prods or sticks, and overall bruising was reduced compared with a previous study in 2008. In the European Union, both legislation, such as Regulation (EC) 1/2005 on the protection of animals during transport and related operations, and assurance scheme requirements have improved conditions during transit. It is important that surveillance data, such as mortality on long journeys, are analysed to drive further improvements. The outcome of just such a paper, which evaluated records from an Italian control post, indicated that high stocking densities of sheep/goats transported by road on long journeys are associated with increased morbidity and mortality. Several papers explore aspects of catching and transport for different species and genotypes, investigating specific stressors, methods of identifying them, and their consequences (including for meat quality). A review paper considers mobile poultry processing units as an option for small-scale production, and the concluding paper investigates ways of cleaning poultry transport coops to minimise cross-contamination. The Special Issue, therefore, adds to the body of evidence that can be drawn upon to reduce the negative consequences of farm animal transport. It is increasingly evident that to achieve both good animal welfare and economic reward in practice requires both “ownership” and attention to detail throughout the whole chain from farm to slaughter. This likely will be driven by increased consumer awareness demanding assurance. Hopefully public awareness will also end the unnecessary long-distance transport of cattle and sheep by sea from Australia to the Middle East for slaughter, which is associated with high mortality and much suffering in overcrowded, hot, and humid conditions.

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Review

Welfare Problems in Cattle, Pigs, and Sheep that Persist Even Though Scientific Research Clearly Shows How to Prevent Them

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Simple Summary: Great strides have been made to improve animal welfare. Unfortunately, there are certain problems that continue to persist. The causes of these problems range from a lack of financial accountability for losses, failure to measure them, or repeating old mistakes. Some examples of persistent problems are bruises, failure to vaccinate cattle, and high percentages of lame livestock. Both good management practices and providing the right financial incentives will improve welfare.

Abstract: Poor production and handling practices continue to persist that are both detrimental to animal welfare and financially burdensome. These practices continue to persist for three reasons: (1) a segmented marketing chain where a producer is not held financially accountable for losses; (2) failure to measure and assess chronic painful problems such as lame livestock; and (3) repeating old mistakes, such as housing fattening cattle for long periods of time on bare concrete. Two examples of the first type of losses are bruises caused by poor handling and sick cattle at feedlots caused by failure to vaccinate and precondition weaned calves at the farm of origin. In some segmented marketing systems, there is no economic incentive to vaccinate. When the animals get sick, the responsibility gets passed to the next person. Buyers of meat products can reduce these "passed on" losses by source verification. The first step to reducing problems, such as lame livestock, is to measure the percentage of lame animals and work with the producers to reduce them. Also, transportation payments should be changed and contracts should be based on the condition of the animals at delivery.

Keywords: welfare; cattle; swine; lameness; at risk cattle; bovine respiratory disease

1. Introduction

Great strides have been made in animal welfare research. Both scientific studies and practical experience clearly show that best practices in both stockmanship and housing will produce more productive animals [1,2]. Unfortunately, some producers do not follow the recommendations made by researchers. Why do certain bad practices still persist today when they are detrimental to animal welfare and cost money? The poor practices continue even though producers know they are wrong because there is not a sufficient financial incentive to stop doing them. For example, bruises on beef cattle cost the cattle industry millions of dollars worldwide. Poor handling and transportation practices are a major cause of bruises. Improving cattle handling and transportation practices can reduce the percentage of carcasses with bruises [3,4]. Another problem that has resurfaced in recent years is raising fed cattle on concrete slats which results in swollen joints [5]. This causes problems with excessive electric goad use at the abattoir. Under EU legislation, severely lame animals cannot be transported. In the United States, there are no regulations to prohibit transport of severely lame animals.

The practice of shipping weaned calves from pasture to feedlots with no vaccinations still occurs today. This is a bad practice that will increase both morbidity and mortality [6,7]. Research clearly shows that vaccinating and weaning beef calves 45–60 days before they leave the ranch of origin will reduce sickness and death losses [8,9]. Vaccinating calves on the day they are shipped is worthless. The vaccine will not have time to create immunity before the calves are exposed to pathogens at the feedlot. It has been suggested that the feedlot veterinarians should make vaccinations a requirement before entry. The United States has very little legislation regarding this matter and it would be difficult to implement anyway. If one feedlot required preconditioning and vaccinations, the producer may send the calves to a feedlot that does not have this requirement.

Lameness is another serious issue in dairy cows, pigs, and poultry. Lame animals are less productive [10]. There are some dairies that do an excellent job of preventing their cows from becoming lame and others with high percentages of lame cows. Research clearly shows that there are huge differences in the percentage of lame cows between the best dairies and the worst dairies [11,12].

The purpose of this paper is to discuss why these problems continue to persist and address some ways to remedy these problems. There are four major causes of these persistent animal welfare problems. They are: (1) lack of financial accountability for losses; (2) poor management; (3) wrong financial incentives; and (4) “bad becoming normal”. The latter occurs when conditions deteriorate slowly and people do not perceive the problem.

2. Repeating an Old Mistake—Lame Cattle Housed on Concrete

High percentages of lame cattle is a good example of bad becoming normal. In the United States, problems with lameness in fed steers and heifers have recently increased. This is partly due to expansion of the ethanol industry, which has provided a financial motivation to feed cattle in the Midwest close to where the corn (maize) is grown. Wet distillers grain from the ethanol industry is fed to cattle. Since this feedstuff is expensive to ship, cattle that had previously been fed in dry western dirt feedlots are now remaining in the midwestern United States. Due to high rainfall, some cattle feeders are now fattening cattle on bare concrete floors without bedding. Dirt feedlots in the Midwest become extremely muddy due to high rainfall. The good cattle feeders provide bedding in the concrete pens, but some producers raise cattle on bare concrete. This can result in both swollen knee joints [5] and lameness. There have been reports from people working in abattoirs that the worst lame fed cattle arrive during the night shift. Managers are less likely to see them at this time. Cattle that are reluctant to move are more likely to be abused with electric goads. In the 1970s, the author observed three slatted feedlots with bare concrete slats. In all three feedlots, swollen joints and lameness started occurring after the animals had gained 225 kg on the slats. A rubber surface placed on the slats slows down the development of swollen joints [5], but it does not eliminate leg injuries. Unfortunately, some US cattle feeders are now repeating the same mistakes they made in the 1970s. At that time, huge feedlots with concrete slatted floors were built in the United States. During the 1980s, they were phased out due to lameness, other health issues, and high costs. The author has been in the beef industry for over 40 years. New people coming into the industry are reinventing old bad mistakes because they do not know about them.

The author has also observed recent problems with lame cattle in other countries that house fattening cattle on bare concrete. High rainfall or scarce bedding materials are the reason for this poor practice. The practice persists because there is no economic incentive to either provide bedding or shorten the length of time that the cattle are kept on bare concrete.

3. No Financial Accountability for Bruised, Dead, or Sick Cattle

Failure to vaccinate weaned beef calves before they leave the ranch, bruises caused by rough handling, and overloading of trucks are often caused by a lack of accountability for losses. Cattle that are moved through auctions or are handled poorly have more bruises than cattle sold directly from the farm to abattoir [13–15]. In this segmented market, no one is held financially accountable for bruises. When producers have to pay for bruises, they can be greatly reduced [3].

In the US cattle industry, a rancher who fails to vaccinate his or her weaned calves is often not held financially accountable if unvaccinated calves get sick or die at the feedlot. The calves often pass through a segmented market chain which usually consists of a local auction, order buyer (dealer), and a final destination at a feedlot. The calves often cross state lines and there is no Federal legislation for individual identification. In some countries, truckers are paid based on how many kilograms of livestock they can load onto a truck [16]. This provides a financial incentive to overload the truck.

4. Methods to Improve Financial Accountability

The first step is for major retail and restaurant buyers to require source-verified livestock. This will make it possible for buyers to provide either financial rewards or fines for high levels of bruises or sick calves. Source verification back to the farm of origin is essential for the effective use of financial incentives. In Europe, legislated source verification procedures make it easier to trace animals back to the farm of origin. Many countries do not have legislated source verification. In the United States, progressive buyers have set up private programs to purchase only source-verified cattle. These animals will have been produced according to the buyer's welfare and production guidelines [16]. In some states, special auctions are used to sell cattle to feedlots that have been verified as prevaccinated. Depending upon cattle-availability market prices, special sales of properly preconditioned cattle do not always result in higher prices for producers [17]. The special preconditioned auctions market the calves to cattle feedlots.

These voluntary private special auctions work well when cattle supplies are plentiful. They are less effective when cattle are scarce because cattle feeders need to fill up their yards. When this happens, greater numbers of "at-risk" cattle are sold at auctions. At-risk cattle is a euphemism for poorly managed calves which have not been prevaccinated. After arrival at the feedlot, the mass treating of at-risk cattle with antibiotics will lower death losses [18]. This improves the welfare of the calves but it is a poor practice due to increasing concerns about antibiotic resistance.

To improve accountability for losses, the author suggests the following recommendations:

(1) High-welfare schemes, such as RSPCA Freedom Foods in the United Kingdom and Niman Ranch and American Humane Certified in the United States, require producers to perform best practices such as vaccinating calves before shipment from the ranch. Producers are encouraged to join these programs to get financial premiums. These programs are monitored by auditors who are independent from the livestock industry.
(2) Retail and restaurant buyers of meat and dairy products should increase the purchase of livestock and poultry that can be source verified back to the farm of origin.
(3) Abattoir management should provide both rewards and fines to reduce bruises and death losses. The author observed a great reduction in downed nonambulatory pigs arriving at an abattoir after initiating a $25 handling fee for each nonambulatory pig.
(4) Change transportation payment and contracts based on the condition of the animals at delivery. Never use contracts based on the number of animals loaded at the farm of origin.

5. Problems Caused by Poor Management

When the author first started her career in the 1970s, she mistakenly thought that newer, more advanced equipment could be designed that would prevent poor handling. Equipment is only half the equation and good management is the other half. Too often people want the wonderful new technology that they think will solve all their problems. The author has a saying that "People want the thing more than the management". New technology does not replace the need for good management. The purchase of a new technology, such as a fancy milking equipment, a new truck, or a computer, does not replace management. Purchase of a new technology is a one-time expense, whereas good management and stockmanship require continuous attention to many small details. Some of the details of procedure that require monitoring during handling are the number of animals moved in each group, electric goad use, and training employees to use behavioral principles of livestock handling.

6. Importance of Stockmanship

Research studies clearly show the benefits of good stockmanship. Fearful animals with large flight zones that avoid people are less productive [19–21]. During the last 10 years, the use of training programs to improve stockmanship has increased. Two research studies in the United States showed that cattle handling in large feedlots had improved [21,22]. A study done in California on ranches showed that ranchers who had Beef Quality Assurance training had better handling scores [23,24].

7. Ways to Improve Management

(1) Managers must care about animal welfare. Top managers who do not care will have problems in their operations. Audits of abattoirs showed that conditions improved when poor managers were removed [25].
(2) Use outcome based numerical standards. Numerical scoring of handling practices greatly improved handling and stunning in slaughter plants when it was implemented by major meat buyers [25,26].
(3) Do not understaff or overwork employees. Tired overworked employees will not care and are more likely to handle animals roughly.
(4) Top managers must get out of the office and regularly observe conditions on farms and abattoirs.
(5) Never pay people who handle livestock on a piecework basis. When a piecework program is used, the employee pay is based on the number of animals handled. Create incentives to reward employees for good practices. Reward employees handling livestock and poultry for low levels of death losses, injuries, and bruising.
(6) Employees and stockpeople should be trained to have a positive attitude towards animals. Research studies clearly show that a positive attitude and liking animals improves productivity [1,27].

8. Bad Becomes Normal

A North American study published in 2012 reported that lameness in dairy cows became so severe that a quarter of all cows were classified as lame [28] and 33% were at risk of becoming lame [29]. This is just one example of bad becoming normal. The percentage of lame cows had slowly increased and it was not noticed by the dairy managers. Studies have also shown that a dairy manager will greatly underestimate the percentage of lame cows [30,31].

The first step in reducing lameness is to start measuring it. Research in Wisconsin has shown great progress in reducing the percentage of lame dairy cows [32]. This was accomplished by measuring lameness on a regular basis plus a comprehensive program of university extension personnel working with dairy managers. Studies have also shown that cows with swollen hocks were more likely to become lame. Good management practices, such as frequent attention to bedding or cubicles (freestalls), will reduce the percentage of cows with swollen hocks [33].

Another example of bad becoming normal is heat stress in large heavy feedlot cattle. Open-mouth breathing in cattle when they are at rest is a sign of severe heat stress [34,35]. Problems with heat stress may have increased because fed cattle are fed to heavier weights, reach market weight at younger ages, and are often black-hided cattle. Other issues may be the use of beta-agonists during hot weather [16,36]. The problem is that managers may get accustomed to looking at panting and think it is like a dog panting. Cattle that are panting are experiencing severe heat stress [34].

The bulldog is not a farm animal, but it is an extreme example of bad becoming normal. The breed standard is verbal. The American Kennel Club Official Standard States a bulldog should have a "massive short faced head, wide shoulders, and sturdy limbs". When a verbal standard is followed, breeders do not know when to stop breeding animals with shorter and shorter noses. There is a picture from a 1938 New York Times article labelled "Bull dog's dilemma". It shows a functional dog. It is totally different from some of today's extreme bulldogs.

9. Methods to Prevent Bad from Becoming Normal

Lameness—Use both lameness scoring systems and leg conformation assessments [37–39]. Continuous measurement will prevent the percentage of lame animals from gradually increasing.

Body Condition Scoring—This will prevent breeding livestock from becoming too thin [40]. For sows, a simple way to assess poor body condition is: if vertebrae bumps show along her back, she is too thin [16]. A sow that is showing vertebrae bumps would be fit for transport and she should be culled from the herd before she becomes unfit.

Injury Scoring and Bruise Scoring—Scoring systems are available for bruises on carcasses [41,42] and for other injuries, such as swollen hocks on dairy cows and confined beef cattle [33]. On sows, shoulder lesions, damage from fighting, foot lesions, and other abnormalities should be scored [43,44].

Livestock Hygiene Scoring—Dirty Animal Scorings—some good information is in Welfare Quality [45,46].

Scoring of Livestock Handling—Some of the variables that are scored are: electric goad use [47], slips and falls [45], and vocalization during handling and turning back or balking during handling [45,48,49]. The advantage of these scoring systems is that they make it possible to determine if practices are improving or becoming worse. Some systematic reviews of animal-based welfare indicators are in [50–52].

10. Abuse of Animals with Little Economic Value

Animals that have little economic value provide no economic incentive for treating them well. Some of the problem areas are old cull breeding stock and newborn bull dairy calves. In the United States, development of a large Holstein fed steer market has increased the value of bull dairy calves. In some parts of the United States, Holsteins are half of the fed cattle market. Dairies are also gradually changing their mindset that cattle are raised for both milk and beef. Developing methods to increase the economic value of an animal will often improve welfare.

11. Conclusions

There are welfare problems that still persist, such as unvaccinated cattle arriving at feedlots and high percentages of lame cattle. The first problem could be remedied by encouraging more producers to enroll in high-welfare programs that require best practices. High percentages of lame dairy cows is an example of bad becoming normal. This occurred because the percentage of lame dairy cows slowly increased without being noticed. The use of numerical scoring of lameness is recommended to help managers reduce lameness in both dairy and beef cattle.

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animals

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Article

"I Feel Sorry for Them": Australian Meat Consumers' Perceptions about Sheep and Beef Cattle Transportation

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Simple Summary: Understanding what concerns about animal welfare are most prominent among members of the public is critical to improve processes in the meat production industry. Hence, this study uses qualitative data to explore how Australian meat consumers viewed one aspect of the production process, livestock transportation. Participants in this study were concerned about the close packing of animals into trucks and ships, and their experiences during long-distance voyages; their views on this topic may be motivated by genuine concerns about animal welfare, together with anthropomorphic tendencies to project human feelings onto these animal experiences and emotional responses, due to transport being associated with slaughter. Given the importance of transport to the Australia red-meat production industry, we argue that public views should be considered as the sector modifies its practices; in addition, higher levels of transparency and communication about practices associated with good animal welfare are needed.

Abstract: Concern for livestock welfare is significantly increasing in many parts of the world. One area of concern is the transportation of livestock. Using qualitative research methods, this research explores the concerns of Australian meat consumers related to livestock transportation practices, both on land by truck and on sea by ship. Participants were predominantly concerned about animals being "crammed" into trucks and ships, and the long distances over which livestock were transported. Likely contributors to these reactions are the high visibility of truck transport in urban areas, and recent media and political attention to the live-export issue in Australia. We argue that participants' concerns about transport are arising for a variety of reasons, including anthropomorphic tendencies, genuine concern for the welfare of farm animals, and emotional responses related to the discomfort experienced by meat consumers when they are reminded of the meat-animal connection. Given the importance of transport to the red-meat production industry, these results suggest that the sector may need to reconsider some of their practices and increase transparency and communication about the practices, which they utilise to ensure good animal welfare.

Keywords: livestock; transport; live export; trucks; road transport; consumer perception; Australia

1. Introduction

1.1. Context of the Study

Increasing public concern for the welfare of animals used for food is well documented in several countries [1–3], including Australia [4–6], and has been attributed to increased awareness of animal sentience [7] alongside a growth in intensification of agriculture and the increasing urbanisation of societies. Taylor and Signal [8] argue that urbanisation has led to a "progressively 'removed' form of knowledge regarding animals [which is] gained through, for example, nature programs and zoos rather than through exposure to primary production processes" [8] (p. 348). The data presented in this paper have been generated in the context of a project that aims to explore Australian meat consumers' attitudes toward farm animal welfare in the Australian sheep and beef cattle industries. In qualitative research, participants sometimes take their responses into unexpected domains or emphasise particular issues much more than researchers might anticipate. In this study, we found that many concerns expressed by participants about red meat production focused on the transportation of livestock in trucks and also by sea as part of Australia's live-export trade (discussed in more detail below). Hence, in this paper we specifically focus on concerns about livestock transport. We were particularly interested in whether participants spontaneously offered explanations of why they were concerned about transport and sought to have participants articulate their reasoning behind the explanations that they provided.

1.2. Human-Animal Relations and Livestock Production

Sociological research exploring human-animal relations has highlighted changes in public attitudes toward animals over the past 40 to 50 years, with an evolution from a traditional utilitarian perspective to a more compassionate and empathetic level of care. This change has been attributed to the growth of post-material values within Western societies more generally [9–12]. These changes in human-animal relations have resulted in increased tendencies to anthropomorphise, that is, to attribute human mental states (thoughts, feelings, motivations and beliefs) to non-human objects or creatures [13]. Many now view animals as moral others who are perceived to have distinct emotions and personalities [14]. Jasper and Nelkin [15] suggest that these changes in attitudes toward animals are a result of "sentimental anthropomorphism", which stems from increasing urbanisation and a shift in focus from considering animals to be part of food production to viewing animals as increasingly meaningful members of society and/or the family. In the context of livestock production, when the characteristics of the production system are perceived to cause harm or suffering to animals, for example, because the free will of the animal is taken away, the specific conditions responsible for the suffering are likely to be perceived as immoral [16] and disgusting [17,18], particularly as people tend to anthropomorphise and consider how the animal is likely to be feeling in its current situation. As a result, many studies have demonstrated that people have become increasingly concerned about intensive animal agriculture, and that they prefer animals to be reared in free-range systems, which allow them to express "natural behaviours" [1,2,18,19].

1.3. Previous Work on Consumer Perceptions of Livestock Transport

Long distance transport of livestock is common in many countries, due to the low cost of transport relative to market value, market demand and relative seasonal variation, geographical concentration of production, and contract agreements [20,21]. Some researchers working in locales other than Australia have previously argued that people with little or no connection to agriculture lack knowledge about the practices associated with livestock transportation and animal welfare considerations during transport [22–24], and thus, many tend to believe that minimum to no transportation during an animal's life is essential to good animal welfare [25]. Previous research from Europe has revealed that space allowance, shockproof and calm transportation methods, and duration of transport were of most concern to participants [1]. Similarly, long distances travelled to slaughter have also proved problematic for Canadians [2].

1.4. Livestock Transport in Australia

Australia is a large country and livestock may be required to be moved long distances to meat processors or to ports for export [21], and there are clear standards in Australia to help ensure animal welfare during transportation. Within the transportation guidelines as published by Animal Health Australia [26], it is indicated that animals should not be loaded too tightly or too loosely to minimise the risk of animal injury, and that internal trailer gates should be closed during transportation to ensure that the stocking density is evenly distributed within the trailer. The guidelines also state that cattle older than six months and sheep older than four months must not be off water for more than 48 h, which in turn generates the basis for the allowable travelling time.

One of the most visible parts of the sector where Australian livestock are subject to long distance transport is within the highly contentious live export trade [27], which is currently one of the most debated issues in association with livestock welfare in Australia. Debates over and campaigns against live export have been long-standing, resulting in the 2011 suspension of the cattle trade to Indonesia [28,29], and subsequent resumption of the trade, which received prominent coverage in the Australian media [28,30–32]. This media coverage also resulted in many people expressing outrage and campaigning against the trade on social media [33–35]. Research specifically into attitudes to the live export trade reveal that a majority of the Australian public does not support the trade [27,30].

1.5. Research Objectives and Approach

The study on which this paper is based endeavoured to explore the understandings of meat consumers about the welfare of sheep and beef cattle in Australia. While transportation of livestock is a major part of Australian livestock production, there is limited information about how Australian meat consumers feel about livestock transportation practices outside of the context of live export. Participants in our project were not asked explicitly about particular production practices; instead we used open-ended, general lines of questioning to form deeper understandings of what was of most concern to participants about farm animal welfare.

Our methods are based on the generic inductive qualitative model [36,37], which combines the data-gathering processes with description and interpretation while establishing research questions, along with purposeful sampling, including demographic-based recruitment. This approach strengthens our abilities to generalise, for example across populations and to other locations. However, this approach does not seek to generate statistically significant data or strict representativeness, due to the qualitative nature of the research methods utilised [37,38], which put greater emphasis on what participants say rather than how many participants made one particular claim.

In this paper, we use thematic analysis of interviews and focus groups to examine Australian meat consumers' attitudes toward livestock transportation. Unlike in some previous qualitative studies (for example Clark et al. [39]), concerns associated with the transportation of livestock within the current research arose spontaneously. We contend that transport was of particular concern for our participants, due to the visibility of truck transport within the locations where interviews and focus groups were conducted. Livestock trucks are commonplace on major thoroughfares in Adelaide and Toowoomba, and to a lesser degree in Melbourne, which were our three locations for data gathering. In relation to livestock transportation by ship, the issue of live export has been an extremely contentious public issue in Australia, which has received significant media and political attention. Due to the open nature of questioning used in the current study, we were able to explore why transport was of particular concern for participants. Our findings suggest that consumers are concerned about animals being tightly packed or "crammed" into trucks and live export vessels and transported long distances particularly to slaughter, though there are several reasons that might underlie these concerns. We conclude that our findings suggest that the animal meat sector may need to reconsider some of their practices to align them more closely with community expectations along with current animal welfare science, and that they should increase transparency and communication about the practices, which they utilise to ensure good animal welfare, particularly those associated with transport.

2. Materials and Methods

This research was approved by the University of Adelaide's Human Research Ethics committee (H-2012-262) and conducted according to Australian national guidelines [40]. Participants were required to be over the age of 18 and regular consumers of meat to participate in this research. Overall, 66 meat consumers from across Australia participated in the research during 2015 and 2016. Of these participants, 67% were women. Participants' ages ranged from 18–24 years to over 65.

The research was conducted in Adelaide, South Australia (population of approximately 1.2 million), Melbourne, Victoria (population of approximately 4.65 million), and Toowoomba, Queensland (population of approximately 115,000). Focus groups and "mall-intercept" interviews [41] were used to collect data, with the latter utilised primarily to provide a balance in the overall sample in terms of demographics, particularly socioeconomic status. Three focus groups were conducted (2 in Adelaide, 1 in Melbourne) with 9 participants in each group (a total of 27 participants (40.9%)). The remaining 39 participants (59.1%) were involved in mall-intercept interviews. Focus group participants were recruited through community and social media announcements and flyers distributed at public events.

Focus groups and interviews were structured and included a series of discussion points about the welfare of sheep and beef cattle more broadly; however, this paper focuses on the concerns expressed by participants about the welfare of animals during transportation by truck and ship. Questions directed at participants were structured to remain open ended to allow them to express their own thoughts and use their own preferred concepts and language, rather than restricting their responses by providing a series of predetermined answers from which to select. The use of open-ended questions without prompted answers enabled us to develop a greater understanding about which issues were most important to the participants and also allowed them to reflect on their experiential knowledge [42]. Asking the same basic questions in each focus group and of each participant provided a foundation to aid comparison of results.

Focus groups and interviews were digitally recorded, and then transcribed fully, checked for accuracy against handwritten notes and anonymised. Each transcript was treated as a rich, narrative text and the first author inductively coded all open responses in NVivo [43] using methods similar to 'open-coding' [44]. These codes were subsequently reorganised, combined, and relabelled into a smaller number of general themes through a process of constant comparison. The final themes were later organised under overarching categories, and illustrative examples selected for reporting the findings. Measures of trustworthiness and rigour included comparison of coding of a subset of transcripts with the second author and discussion of all coding and themes with all authors. Quotes used in the results and discussion are illustrative and are typical of those coded to the themes identified during analysis.

3. Results

In this section, we briefly present evidence from our data based on participants' responses, organised by key themes. We analyse these data in more detail in the discussion.

3.1. Transport as Compared to Other Concerns

As mentioned previously, participant responses to open questions about areas of concern within the red meat production sector highlighted that transport was a key area that was considered to impact negatively on farm animal welfare. This concern was particularly apparent in the context of the responses to the initial orientation question, which asked participants to describe the images that come to mind when they think about beef cattle or meat sheep farming, as illustrated below:

> Researcher: So when I ask you to get a picture in your mind about animal farming, beef or sheep cattle what kind of images come to your mind?
>
> US3: Umm what disturbs me is animal transport
>
> Researcher: Right
>
> US3: So I just see animals packed into vehicles

Researcher: So that is what you see here in the streets of Adelaide or are you talking about what you see on television?

US3: Uh no what I see because I live at Crafers [A suburb in the foothills east of Adelaide and connected to the Central Business District (downtown) by a freeway that connects the city with farmland and a large abattoir] and I am up and down the freeway regularly so we pass next to or behind the vehicle like that. I do find it quite distressing.

Researcher: When you think of cattle farming in your head, what kind of images do you see?

PFG8: I think of umm the sheep in the uhh trucks, all shoved in there, that's what I think of yeah. On Portrush Road [This road is a major north-south thoroughfare in Adelaide that also runs through high SES suburbs] yeah.

Researcher: So when I say sheep and beef farming, what do you think of? What sorts of images come to mind?

SB4: Oh, crowded cattle trucks, you know, animals being badly bloody treated actually.

Researcher: Can you elaborate a bit more on that?

SB4: You know, you see some cattle trucks sometimes, they're absolutely, animals crammed in, you know? You see the live beef export, that's disgusting. You see the ships being loaded up with sheep and that, I don't know if they still do live sheep exports. And I've seen some of that . . . documentary, I forget what they call it now but it showed film, belting the crap out of them so yeah I just think of cruelty.

Researcher: When I talk about sheep and beef farming, what sort of pictures come to your head?

GB1: Sheep and beef farming. Well I see the sheep out in the paddock [pause] . . . I don't like the way they get pushed into the trucks.

Researcher: You don't like the trucks?

GB1: No. They're pushed in, they push, push, just how many get in? Then I, what I don't like when they get transported.

Themes emerging from the data reveal that the participants in this research described transport in negative terms, such as "cruelty", and used emotional language, such as "distress" and "disgust", to express their perceptions of transport that they both witnessed first-hand, as well as via media representations. In summary, participants perceived that various aspects of livestock transportation had a negative impact on animal welfare and were concerned. As there were notable differences in the way participants described transport via truck and transport via ship, the following sections examine these modes of transport separately.

3.2. Perceptions of Transport via Truck

As seen in the quotes above, transport by truck was described in emotional language by participants. The predominant concern was the idea that animals are "crammed", "shoved", or forced into trucks, with limited space to move, and that this was "bad treatment".

Other participants described transport in terms of the emotions expressed by the animals being transported, as illustrated below:

> US2: It really troubles me on a really hot day when you drive down the road and there is a cattle van or a chicken truck and it's a really hot day and they are in a metal van and they're all just crammed in against the hot walls . . . they look troubled and you know you see them sniffing at the side of the thing and it's horrible. It makes me really, really sad.

This quote also illustrates participants' concerns about the impacts of exposure to the environment on farm animal welfare during transportation. In addition, participants perceived that there were risks of injury during transportation:

> GB1: . . . [I'm] not happy when they get pushed into trucks, especially up I think in the Northern Territory well where there's big trucks and . . . sometimes the leg hangs out and I don't like the way they get transported.

Participants were also concerned about the impacts of long-distance transportation on animal welfare and linked these impacts to the resulting meat quality:

> Researcher: You mentioned ah slaughter . . . are there any aspects of that that concern you more than others?

> MFG9: Ah yeah, quite a lot, particularly in Victoria because we have very structured system which makes it very difficult for farmers to, for instance, go to a smaller abattoir. A lot of time they are forced to take the animals long distances which often means putting them into, jamming them into trucks and putting the animals under an enormous amount of stress umm which I think is bad for the animals, it is bad for the meat.

In summary, participants expressed concerns about animals being tightly packed for transportation via truck and associated transportation with exposure to the environment and risk of injury, both of which they viewed as impacting negatively on animal welfare.

3.3. Perceptions of Transport via Ship

Similar to perceptions of truck transport, participants expressed disgust and concern for animal suffering when animals were tightly packed on live export vessels and transported long distances:

> Researcher: You don't like live export?

> UB1: Not really. Have you ever been on one of the ships? It is absolutely foul.

> Researcher: Are you worried about the animals on the [live export] trip or about what happens to them when they get to the other end?

> SS2: Both because you know they're packed so tightly and haven't got water and access to. I don't know. I just know it's a long journey and it's cruelty and they suffer.

Participants were not only worried about the transportation process, but also expressed concerns about the destination countries, often voicing fears about how the animals are treated in countries who receive livestock through the live export supply chain. Concerns about animal treatment in receiving countries were generally associated by participants with halal slaughter practices as most countries which receive live animals from Australia have a Islamic majority.

> Researcher: Are there any issues you are unsure about associated with beef and sheep production?

> SB2: I am only unsure of when the sheep or livestock is shipped overseas. To say for instance to Indonesia or any Asian country or anywhere, how they are treated there. I have got a bit of a thing about that because there has been programs on TV . . . [talking about] the inhumane attitude they have toward these animals. So it is a concern, I must admit.

Many participants advocated for the live export industry to be closed, often suggesting that processing in Australia would be a better option.

> UB3: I feel that the live animal export is a major issue as far as I am concerned personally and everyone in my circles. I think we can do far more for this country by bringing onshore the abattoirs and processing situations and if [other] countries insist on having our live animals then I am afraid it should be a closed door and we seek other markets that do accept our processed meat.

Ultimately, participants believed the live export trade to be unnecessary and often questioned why animals are subject to poor conditions on ships when in their views other viable alternatives exist within the Australian meat processing sector.

3.4. Transporters as the Problematic 'Middle Men'

Participants' perceptions revealed that they viewed transportation as a distinct stage in the production of red meat and one that was beyond the 'care' of farmers. Transporters were viewed as 'middle men', and were considered to include those working in the parts of the value chain between farmers and consumers, those involved in the sale of livestock in saleyards, and arguably even those in the retail sector as revealed in the quotes below:

> Researcher: Do you think farmers do enough to ensure good animal welfare?

> UB3: Umm we are talking about sheep and cattle, yes. But it is after that when they go out to the live trade umm that worries me. In the trucks, that worries me. Once they leave the farm gate I am concerned. The third, the second, the person in the middle. The middle man before the abattoir I really care about . . .

> SB2: That is what it is all about. It's all a business. It is the second and third man that make [agricultural] business a problem.

3.5. Transport to Slaughter

The fact that animals in livestock trucks were being transported to slaughter also impacted on participants' perceptions of transportation. Participants expressed that there is a special responsibility on the part of transporters and others to ensure good welfare because animals were travelling to "their deaths", as exemplified by the following quotes:

> SS3: Umm I just think of the animals and I feel sorry for them because ultimately they are going to be killed and that's, what, emotionally I think ohh that isn't good.

> US2: I think the way that they are transported from their farm or their shed or wherever they are coming from to go to the place they are killed, some thought needs to be put into that as well . . . So I feel like they're, if it could be just one bad day, I guess that's nice but making sure that day is not painful and unpleasant as possible.

These quotes demonstrate that participants respond emotionally to the idea of animals travelling to slaughter and believe that animals should be treated particularly well during this process.

4. Discussion: Why is Transport So Problematic?

4.1. Visibility

Transport was a key area of concern when participants described their perceptions of red meat consumption. Transport may be of greater concern to the general public than other practices affecting farm animal welfare, for example painful practices in animal care and production, because transport is highly visible to the public, due to livestock trucks passing regularly through urban areas. Participants (in Adelaide and Toowoomba in particular) mentioned seeing trucks in their neighbourhoods and on main roads. For many in urban areas, the only occasion on which they see meat producing animals may well be during transport. Areas of beef cattle and sheep meat production are often remote from urban areas, but in smaller cities the rationalisation of the meat processing sector means that animals may need to be transported from one side of the city to the other, with the shortest route being through urban areas.

In addition to the first-hand observations of animals in trucks in urban areas, the live export industry has frequently been covered in the Australia press in recent years [45,46] following on from exposés revealing abuse in destination countries, appalling conditions during transport, and continued breaches of industry standards. It should be noted that we are not suggesting that the media coverage of live export is influencing public perceptions more generally of transportation, only that the issue of animal transport has been prominent in public discourse in Australia in recent years via the live export debates. The perceptions of live export expressed by the participants in this study are consistent with those documented in other scholarship [27,30] and include "disgust", outrage, and calls for the trade to be discontinued, as we discuss in more detail below.

4.2. The Role of Anthropomorphism

Concerns expressed in the current study about animals being tightly packed into trucks or ships may result from participants projecting their emotions onto the animals based on the participants' own experiences in crowded situations. Crowding is a subjective psychological response to density and refers to situations which result in feelings of restriction within the individuals exposed to limited or tight spaces [47,48]. In relation to human population density, different cultures have different levels of adaptation and tolerance to crowding [48]. Tolerance of crowding is related to socioeconomic background, age, education, culture, and previous living environments. People who have previously lived in a high density, crowded environment are less likely to feel crowded than someone who lives in a more spacious environment [48–50]. In Australia, we have relatively low population density relative to other countries; however, Australians are still likely to have experienced situations of crowding, such as at a sporting event or music concert and thus can still imagine discomfort at being crowded or "crammed" into a small space. Long distance transport could be viewed in the same way: Many people do not like to travel in tight spaces, such as planes or cars, for long periods or distances. In short, concern about the density at which animals are loaded for transportation may be reflections of the participants' own feelings about crowding and confinement.

4.3. What about Disgust and Moral Outrage?

Participants frequently described their perceptions of livestock transport as 'disgusting'. Several authors have suggested links between disgust and moral outrage [51–54]. Disgust is considered to be a moral emotion [55] and a "gut feeling" [56], which is provoked by something, or the thought of something, violating the purity of the body or soul [57]. The feeling of disgust has previously been identified as resulting from imagery of animals raised under 'cruel' factory farming conditions [17,18]. Some authors suggest that people find things more immoral when they are exposed to disgust cues (such as the presence of contaminants, for example faeces) [56]. Therefore, it may be that the current study's participants found what they viewed as the crowded and potentially unsanitary conditions experienced by animals during transport to be disgusting and hence morally problematic. This disgust may also be connected to the idea that these animals will eventually be consumed, as disgust often arises in connection to issues relating to food consumption [58–60]. Alternatively, participants may have found the treatment of animals during transport to be morally problematic (because it is "cruel") and hence "disgusting". It is difficult to disentangle these concepts or to definitively determine the causality between them, given their tight relationship in our participants' responses.

4.4. The Connection of Transport to Slaughter

The visibility of animals in trucks (or on ships in the media or in person) may serve as a reminder that animals are killed to produce meat and hence may reinforce the meat-animal connection, which may be distressing even for meat consumers. In Australia, meat is typically purchased from the supermarket or a butcher's shop in pre-packaged forms and generally lacks much resemblance to the live animal that it once was [61,62]. Disguising the animal characteristics of meat by removing prompts, such as the head, feet, and skin, removes the associated personality and intelligence of the living animal, which further enables the de-animalising process [63]. Supermarkets have contributed to this process by presenting ready-cooked meat in packages, which makes the original animal even more distant from products with uncooked animal flesh, which arguably has more transparency with regard to its origins [64]. The distinction between the animal and meat produced from it is further complicated through creation of semantic differences particularly in English, which eliminates animal designators, such as cow and sheep, by substituting terms, such as beef and lamb in the context of consumption [63]. Such differentiation reinforces the de-animalising process, which allows consumers to generate mental distinctions between the living animal and meat product, and hence detach the one from the other. As Leroy and Praet [65] describe, even with an increasing level of moral aversion toward animal killing, those in modern societies are still fond of consuming meat, though they are experiencing increasing levels of ambivalence.

4.5. Genuine Concern for the Welfare of Animals

Negative emotions toward livestock transport highlighted in the current study appear to be related to animals being "crammed" into trailers or ships, which participants felt was cruel and inhumane. Australia has one of the highest rates of pet ownership globally, with 62 percent of households owning pets [66]. Many Australian families have become "more-than-human" [67] with a higher proportion of Australians living with a dog and/or cat than with a child [68]. Alongside the increase in pet ownership, there has been an incredible economic rise in the pet industry, with Australian households spending more than $12.2 billion annually on pet products and services, representing an increase of 42 percent from 2013 to 2016 [69].

Australians' increasing concerns about farm animal welfare can also be seen in the response to recent activists' campaigns about caged hens [70] and the live export issue [17,28]. In activist campaign videos, animals are often represented as individuals (frequently with names and personalities) who are suffering. The activist organisation Animals Australia has been at the forefront of broadcasting moments of "extreme cruelty experienced by these animals in their last moments" in both domestic and overseas abattoirs with such footage described as "effective enough in mobilising Australians for abattoirs to be shut down . . . and the installation of surveillance cameras in abattoirs" [17] (p. 45), as well as the suspension of live export trade [28]. Despite evidence which suggests that Australian meat consumers typically ignore animal welfare activism, particularly when it is online [38], it is clear that animal welfare activism has played a significant role in raising general awareness and communicating to the public about intensive animal agriculture and the live export controversy.

In the case of live export in particular, many Australians wish to see the trade end [27]. As live export has been a long-standing and ongoing point of contention in Australia, it is unsurprising that it was raised as an issue associated with animal welfare by participants in the current study. Many participants stated that the trade is disgusting and unnecessary, as processing the livestock in Australia and exporting the frozen meat was considered to be more likely to be beneficial to the Australian workforce and economy than are current practices associated exporting the live animal. However, it is unclear whether such an approach would be viable, given that Australia's main export markets require halal slaughter and limited time between slaughter and consumption, and also often do not have extensive refrigeration networks.

4.6. Transport Workers are Not Trusted

Although farmers are generally trusted in Australia to care for the welfare of their animals [71,72], this trust is not extended to those who transport animals. Coleman et al. [73] have demonstrated that 24 percent of the general public in Australia have low trust in workers involved in land-based livestock transport, and 41 percent indicated to have low trust in workers involved in livestock transport by sea. Similar results were found in the current study, with participants often suggesting those involved between the farm gate and the processing plant (i.e., the livestock transporters) are of most concern. Given these public concerns, the industry may need to consider ways to limit or eliminate long distance truck transport by opening, or in the case of Australia re-opening, smaller more localised processing facilities or utilising mobile abattoirs [2]. However, these options may be unrealistic based on the efficiencies obtained by utilising larger processing plants, as well as the high cost of livestock processing in Australia.

5. Conclusions

This paper documents qualitative data that indicates that livestock transport is a key area of concern for Australian meat consumers and is viewed as having negative impacts on animal welfare. The spontaneous responses provided by participants indicate that transport is more prominent among the concerns of meat consumers than other practices that may impact negatively on animal welfare, such as painful procedures. Participants used emotive language to describe their perceptions of transport, including "disgust" and "sadness", and the idea that animals were treated "cruelly" during transport. Negative associations with animals being closely packed together during transport was a strong theme that emerged from the data. Animals themselves were also described as appearing "stressed" and "troubled" during transport. We contend that the high visibility of truck transport in urban areas, and the media and political attention given to the live-export issue in Australia, are likely contributors to the prominence of transport as an area of concern. We also suggest that this concern may be arising for a variety of reasons, including, due to anthropomorphic tendencies, genuine concern for the welfare of farm animals, and/or emotional responses related to the discomfort experienced by meat consumers when they are reminded of the meat-animal connection, given that animals being transported are on their way to a slaughter facility.

The evidence from our work and research by others [19] suggests that the livestock transport sector has to some extent lost consumer trust. We did not anticipate that transport would be as prominent in our research as it in fact was, nor did we expect to document the high visibility of livestock transport trucks in urban areas as described by our participants. Given the importance of transport to the red-meat production industry, these results suggest that the sector may need to reconsider some of their practices so that they are more in line with community expectations and with current animal welfare science, and to increase transparency and communication about the practices, which they utilise to ensure good animal welfare.

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animals

Article

Relationship between Methods of Loading and Unloading, Carcass Bruising, and Animal Welfare in the Transportation of Extensively Reared Beef Cattle

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Simple Summary: In Uruguay, extensive, welfare-friendly beef production is a substantial part of the economy and culture. Transport of beef cattle to the slaughterhouse compromises animal welfare. The objective of this study was to assess transport conditions related to carcass bruising. A total of 242 trucks with 8132 animals were assessed on loading, transport, unloading conditions, and carcass bruising. In 39.3% of the loadings only a flag was used. The fastest unloading time was performed using a flag only. Carcass bruises were assessed by trained observers inside the plant. Although the number of bruises was high, there were no grade 3 bruises, the deepest and severe ones. It appeared that animal welfare training of truck drivers worked out well and the use of flags to move animals increased compared to a previous study in 2008.

Abstract: In Uruguay, extensive, welfare-friendly beef production is a substantial part of the economy and culture. Transport of beef cattle to slaughterhouse compromises animal welfare. The objective of this study was to assess transport conditions related to carcass bruising. A total of 242 trucks with 8132 animals were assessed on loading, transport, unloading conditions, and carcass bruising. Average loading time was 26 min and 21 s and the perception of the truck drivers was correlated with the time took for loading and the use of devices. In 39.3% of the loadings only a flag was used. The average unloading time was 5 min and 54 s with a significant difference in time for the use of devices; only flag 3 min 51 s, cattle prod 6 min 43 s and sticks 8 min 09 s. Of the carcasses observed, 772 (9.5%) had no bruises, 873 (10.7%) had one bruise, 1312 (16.1%) two, 1231 (15.1%) three and 3944 (48.5%) had four or more bruises. Prevalence of bruises were highest on the *Tuber-coxea* (hip) (29.3%) following forequarter (22.4%), *Tuber-ischiadicum* (rear) (17.3%), ribs/flank (14.1%), rump/round (10.1%) and loin (6.8%). Bruises were 68.7% grade 1 and 31.3 % grade 2; there were no grade 3, the deepest ones, observed. It appeared that animal welfare training of truck drivers worked out well and the use of flags increased compared to a previous study in 2008.

Keywords: carcass bruises; cattle transport; animal welfare; extensive production system

1. Introduction

In Uruguay, beef production is an important part of the economy. In 2016, beef was the main export product, and Uruguay is the 6th largest beef exporter in the world, with a US $1000 million income [1]. Worldwide product quality is crucial for the export position, and animal welfare is part of this condition [2]. In the extensive production system used in Uruguay, which is generally welfare friendly, one of the most important stressors for cattle is the transport to the slaughterhouse [3].

These animals are not used to being handled by humans and therefore handling animals on farm, loading and unloading from vehicles, transportation, occasionally passing through livestock markets, and lairage can all affect their welfare [4]. Furthermore, the fasting during transport and stunning at the slaughter affect welfare as well. Reducing physical and emotional stress during transport and associated events can improve both carcass quality and animal welfare [5].

In 1986 welfare was defined by Broom [6] as follows: "The welfare of an individual is its state as regards its attempts to cope with its environment. This includes both the extent of failure to cope and the ease or difficulty in coping". Until the moment of transportation, most beef cattle in Uruguay is reared extensively on pasture or silvopastoral systems. In this situation an animal can adapt to conditions. The deprivation of food and water, fear, arousal, mixing of groups, physical exertion and injuries are all factors that contribute to stress during transport [7]. The inability to adapt to living conditions during transport makes transport a severe impairment in welfare [8,9]. What events contribute most to the loss of welfare, and what measures are most effective to limit this degradation, are not determined yet.

There are two ways carcass bruising influences the value of marketed beef. First, severe bruises are removed from the carcass, resulting in a loss in weight per carcass [10]. Second, the increasing public concern of beef production, the way animals are slaughtered, and the degradation of welfare documented by bruises, can influence the market, and affect total demand for beef. [11]. Economic losses to bruises vary. A study from New-Zealand from 1977 reported 5.58 kg of bruised meat removed from each carcass [12]. The average loss per carcass in Uruguay was found to be 1602 $\pm$ 212 g with a minimum of 50 g and a maximum of 4900 g [10]. Numerous studies have been conducted worldwide to assess the welfare of cattle during transport [13–16]. Physiological parameters, carcass quality and behavioral measures are used for the assessment. Most studies use carcass quality since this is the least invasive method. The physiological responses to handling and transport of different livestock production animals was also extensively documented [9]. Bruising, as an event during transport, increases cortisol, packed cell volume, lactate dehydrogenase isoenzyme and hearth rate, as well as behavior [13–17].

Studies of events that caused bruising were made through direct observations and video analysis of 52 selected cows [18]. They found that 46.1% of the bruises were inflicted during animal-facility interactions, 26.9% from human-animal interactions, and another 26.9% from animal-animal interactions. The potential bruising events occurred mostly during the lairage time (91.2%), and this was confirmed by other authors [19], who added load density and stops during transportation of the cattle as risk factors for bruises. Only bruises on the back were inflicted in the stunning box and most bruises on the pin area were inflicted during loading at the farm [19]. For several years it has been assumed that the welfare of cattle depends greatly on the attitudes and training of stockpersons and on the availability of appropriate facilities [16]. Also, extreme temperatures, below -15 °C or above 20 °C, can influence cattle during transport as well as the experience of the truck driver [13].

It has been reported that factors that were associated with a high number of bruises were bad maintenance of the truck, presence of guillotine doors, journey duration longer than 5 h, bad quality of roads, devices to move animals and the presence of horned animals [3]. The objective of the present study was to evaluate whether certain events during loading, transporting, and unloading cattle have an influence on the amount, location, and degree of bruises on the carcass.

2. Materials and Methods

Over a period of 3 weeks all trucks arriving at one of the major slaughterhouses of Uruguay have been assessed on various parameters in three main subjects: loading, the journey, and unloading. The following day, all animals were assessed on the number, location, and severity of bruises on the carcass according to the method developed previously by Huertas et al. [3]. All assessments were done by four trained observers. To standardize observations in the slaughterhouse, a two-week trial period was performed, in which the inter-observer reliability was optimized. The daily routine of the slaughterhouse was as follows: Most cattle transports arrived between 5 pm and 2 am. After arrival and administration, the truck driver would unload the cattle on a ramp. Animals were given a group number when leaving the truck. This number was related to the farm of origin. Some groups were on multiple trucks and on some trucks there was more than one group present. All groups of animals stayed together and there was no mixing between groups. From this point on the lairage period started in which animals where moved to different pens closer to the stunning box. Slaughter started at 6 a.m. with an all-in-all-out system. Information about the loading and the journey was collected with a survey from the truck drivers.

2.1. Truck, Driver and Loading

The loading of the cattle was assessed from a survey filled in by the truck drivers, asking for: Time the loading took in minutes; the course of loading specified between 'good', 'regular' and 'bad'; the use of the following devices: flags, electric cattle prods and/or sticks. Furthermore, the questionnaire was about the number of years of experience with transporting beef cattle and if an animal welfare training course was attended.

2.2. Unloading

After arrival of the cattle at the slaughterhouse, the course of unloading was assessed. The unloading was scored on way of parking; unloading time in seconds; the behavior of animals on the truck and the behavior when animals were leaving the truck; the use of devices to force animals to move i.e., flags, electric cattle prods and sticks; the manner in which these devices are used i.e., gentle (soft, with a soft touch, just to persuade), intense (stronger than before, but without damaging) or rough (rude, with excessive force, causing damage); the number of people that were involved unloading the cattle and the use of hard shouts and hard sounds. Parking was considered correct if there was <5 cm difference between the trucks and the ramps edge in distance and/or sideward deviation. Behavior was scored and considered nervous if there were one or more animals vocalizing repeatedly, running, and/or jumping. The use of devices to force animals to move i.e., flags, electric cattle prods, and sticks; the way these devices are used i.e., gentle if these were used only as a touch and less than five times, intense if they were used between 5 and 10 times and rough if the devices were used more than ten times and in a hard way per load. The use of sticks usually meant handling a flag as a stick by turning it around or a stick. Shouts were considered used if a person handling the cattle used their voice loudly to urge on cattle. Sounds were assessed similarly, and these were mostly created by hitting devices against the truck.

2.3. Carcass Scoring

At the slaughter line the carcasses were assessed on location and degree of bruises, according to a method developed previously by some authors [3]. Only bruises and lesions with signs of live tissue damage were included. There were three types of lesions distinguished in this study: Grade 1 only superficial tissue is involved, with a diameter less than 10 cm. Grade 2 bruises involved damage to underlying muscle tissue and contained all lesions bigger than 10 cm in diameter. Grade 3 very deep (affecting muscle and even bone), partial or total condemnation of the carcass. Bruises localization: Region 1 is the forequarter, region 2 the ribs and flank, region 3 rump and round, region 4 is the loin. A special category was made for the region of the *Tuber coxae* (hip) and *Tuber-ischiadicum* (rear) i.e., region 5 and 6 respectively. This category was introduced because of the frequent occurrence of lesions. If a bruise or lesion occurred in more than one area the bruise was only recorded in the area where the largest part of that bruise was located.

2.4. Data Analysis

All results were analyzed using IBM SPSS (version 20). For descriptive analyses, different methods have been used. Most continuous variables are described by mean, maximum and minimum. For discrete variables a proportion was determined.

3. Results

In total 242 trucks were used in the analysis. They carried 8132 animals to the slaughterhouse. These were European breeds of an average weight of 450 kg.

3.1. Loading

Average loading time was 26 min and 21 s (from 00:06:00 to 05:00:00) where cattle was loaded from different farms. Course of loading was assessed by the truck drivers, as being good in 81.9%, neutral in 15.7% and bad in 2.4% of cases. According to them, in 10.6% of the loading events no devices (electric cattle prods, sticks or flags) were used, one device in 44.4%, two in 43.2%, three in 10.0%, and four in 2.9% of the events. The devices most used by the drivers to load cattle on the truck were flags (81.7%), sticks (9.1%), electric cattle prods (31.5%) and shouts (49.8%). In 39.3% of the cases only a flag was used as see in Table 1.

Figure 1 shows the trend from which can be concluded that the use of devices such as electric cattle prods and sticks or the use of hard shouts were associated with a neutral or bad course of loading. The use of flags is negatively correlated with the course of loading by the drivers (r = −0.218 p = 0.007) and the use of prods (r = 0.189 p = 0.022) and shouts (r = 0.197 p = 0.022) are positively correlated with the course of loading. The course of loading is expressed as 1 = good/smoothly, without problems; 2 = neutral; 3 = bad/long time needed with many problems.

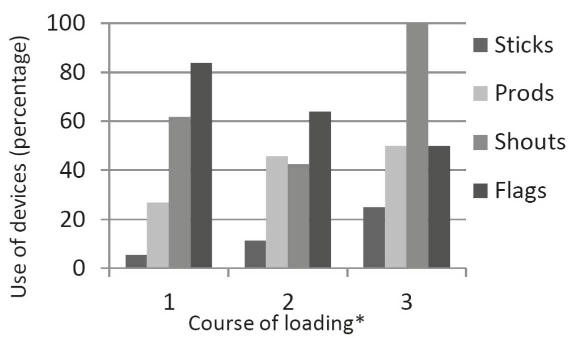

Figure 1. The use of devices in different courses of loading as interpreted by truck driver. *: Interpretation course of loading by truck driver (1 = good, 2 = neutral, 3 = bad).

Table 1. The use of devices when loading cattle.

Device	Number	%
Sticks	1	0.4
Prods	5	2.1
Shouts	6	2.5
Flags	95	39.3
Sticks and prods	1	0.4
Sticks and shouts	4	1.7
Sticks and Flags	2	0.8
Prods and shouts	21	8.7
Prods and Flags	18	7.4
Shouts and Flags	58	24.0
Sticks and Prods and Shouts	7	2.9
Sticks and Prods and Flags	0	0.0
Prods and Shouts and Flags	17	7.0
Sticks and Prods and Shouts and Flags	7	2.9

3.2. Truck Driver

Years of experience transporting cattle was on average 17.4 years with a maximum of 40 years of experience. An animal welfare course was followed by 79.5% of the truck drivers.

3.3. Unloading

Truck parking at the ramp and cattle unloading were observed by the researchers. It was assessed as correctly in 93.7% of all cases. The behavior of the animals on the truck was calm in 69.0% of all cases, when leaving the truck, 80.3% of all cases behaved calm. There was a correlation between the behavior on the truck and when leaving the truck, ($r = 0.294$ $p < 0.000$). The average unloading time was 5 min and 54 s (±03:54) with a minimum of 01:09 and a maximum of 30:21. In 79.6% of unloading events sticks and/or electric cattle prods were used to force animals off the truck as see in Table 2. Hard shouts were used in 59.5% of all cases and hard sounds in 19.4% of all cases. The average number of people interfering with the unloading of cattle was one in 70.7%, two in 26% and three or more in 3.3% of all unloading events.

Table 2. The use of devices when unloading cattle.

Device	Not Used%	Gentle%	Intense%	Rough%
Flag	44.7	15.6	29.1	7.8
Electric prod	44.4	8.3	6.3	41.0
Stick	75.7	8.6	3.3	12.5

In Table 3 the average number of bruises per animal is presented in relation to the various ways of unloading. The correlations between the various ways of unloading and number of bruises per animal were low and not significant.

Table 3. Average number of bruises per animal with the various ways of unloading.

Unloading	AVG Bruises Per Animal
Gentle	3.51
Intense	3.46
Rough	3.70
Flag	3.51
Stick	3.49
Prod	3.64
Noise	3.77
Quiet	3.19

3.4. Carcass Scoring

Of the 8132 carcasses observed, 873 (10.7%) carcasses had one bruise, 1312 (16.1%) had two bruises, 1231 (15.1%) had three bruises and 3944 (48.5%) had four or more bruises. 772 (9.5%) of the carcasses had no bruises. The average number of bruises per carcass was 3.75. The location and prevalence of bruising on a carcass is shown in Table 4. There were no grade 3 bruises in this study.

Table 4. Gradation of bruises and the prevalence of the location on the carcass.

Grade	Forequarter%	Ribs/Flank%	Rump/Round%	Loin%	T. Coxae (hip)%	T. Ischiadicum (rear)%	Total%
1	12.2	7.9	8.3	5.5	20.0	14.9	68.7
2	10.2	6.3	1.9	1.3	9.3	2.4	31.3
Total	22.4	14.1	10.1	6.8	29.3	17.3	100

4. Discussion

There is a close relationship between handling ruminants pre-slaughter and the quantity and quality of the meat they produce [5]. Several initiatives, including activities focused on the impact of pre-slaughter conditions on beef cattle (facilities, equipment, and handling procedures), have been carried out in Uruguay and in the region to promote animal welfare and improve meat quality [20,21]. The results of the present study revealed that the use of a flag to move cattle had the best efficiency. The animals entered the truck quickly and calmly. In 95 (39.3%) out of 242 truckloads only a flag was used to get the animals on the truck. In 58 (24%) cases also shouting was added. This is a substantial difference to the previous findings [3] when in 75% of the cases electric cattle prods were used and in 40% shouting (no flags were used at that time). Electric prods and sticks are used less in the present study. Presumably, the drivers started to use a flag only, but added other devices when the cattle did not enter the truck quickly. These results can be explained, in part, by the diversity of dissemination, extension and training courses on animal welfare concepts and meat quality delivered to the stakeholders [22,23]. However, in the present study, more than 90% of the animals had one or more bruises and this is around 30% more than in the previous study, in which the same scoring system for carcass bruising was used [10].

The extensive way beef cattle are raised in Uruguay is such that animals do not come in contact with humans very often. Transportation is, therefore, very stressful. The use of flags to move cattle is believed to be less traumatic than the use of other devices [24]. Therefore, it was surprising to observe more bruised animals than in the previous study, where flags were not used, but electric cattle prods [3]. Although in the present study, more than 90% of the animals had one or more bruises and this is more than in the past, in which the same scoring system was used for the carcass bruises, there may have been some overvaluation of some bruises by the observers, as found by some authors [25,26]. They found "slight" inter-observer agreement for the number of bruises scored per anatomical site and "fair" for the severity grade of the bruises.

Nevertheless, no bruises of grade 3 were found, the deepest and most severe ones. This finding matches results found recently. The third quality audit of beef was conducted in Uruguay [27] by other authors in the country [28], and no bruises of this degree of severity were observed. It should be noted that in the present study, data were collected on only one slaughterhouse, whereas in the previous studies several abattoirs were visited [3,10,28]. Some researchers state that the presence of carcass bruises is affected by characteristics of the animals, the conditions of transport, the handling of the animals, and the waiting time at the slaughterhouse, more than the loading and unloading process [19,29,30]. Although the presence of horns has been reported as a problem in several countries, the results of some authors [31] suggest that there is no significant relationship between the prevalence of horns and carcass bruising, handling being a critical point. We agree that further research is necessary to evaluate the causality this problem.

When loading cattle, the interpretation of truck drivers regarding handling methods of loading was associated with the duration. A shorter loading time was considered to be good loading. The correlation between the time loading, unloading and the use of different devices is probably due to the fact that people, handling cattle, tended to start using devices if loading and unloading halted. Of all the devices used during unloading, the use of flags only had a significantly lower time unloading cattle.

Interventions at these stages have considered training animal handlers and transporters by showing them the consequences of bad handling with audiovisual material prepared on site. Research results have helped to improve animal welfare and support the development of new legislation or to make changes in the existent legislation related to animal welfare [4,20].

The way to achieve the cultural change necessary to improve animal welfare, operator safety and profitability of the sector is through training and knowledge transfer. The results show that the joint efforts of all the institutions and the active role of the World Organisation for animal health (OIE)

Collaborating Center, a consortium of Chile, Mexico, and Uruguay, have been more effective, as have the continuing education programs implemented by universities [32].

Previous reports and results of this study indicate that the transport of beef cattle to the slaughterhouse is accompanied by impaired animal welfare. A tool to measure animal welfare is to look at bruises on the carcass. These bruises are an incentive to improve the transport conditions and animal welfare because this prevents economic losses. Strict guidelines on human-animal interactions and on the use of devices are important issues. From this point of view, it is good to observe that many drivers used no device (10.6%) or only a flag (39.3%) to load the cattle onto the truck.

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animals

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Review

A Systematic Review of Heat Load in Australian Livestock Transported by Sea

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Simple Summary: The transport of livestock by sea ('live export') is an important contemporary animal welfare issue in Australia. There is particular concern for the effects of heat load on the welfare of sheep being shipped live from Australia to the Middle East during the Northern Hemisphere summer. To reduce bias in a contentious context, we performed a systematic review of the literature relevant to Australian sea transport, heat load, and livestock. We discuss the factors contributing to harmful heat load, pathways for mitigating risks and existing knowledge gaps. We identified several areas requiring research to address these knowledge gaps.

Abstract: The transport of animals by sea ('live export') is one of the most important current animal welfare issues in Australian society. Recent media attention has highlighted concerns regarding the effects of high environmental temperature and humidity on the welfare and mortality of sheep being shipped live from Australia to the Middle East, especially during the Northern Hemisphere summer. To improve understanding of how and why harmful heat load occurs, we systematically reviewed Australian research into heat load and sea transport. High thermal load occurs during the sea transport of sheep and cattle from Australia when animals are subject to hot and humid environmental conditions and cannot remove heat generated by metabolic processes in the body, potentially also gaining heat from the environment. Several approaches have been proposed to mitigate these risks, including avoidance of voyages in hot seasons, selection of heat-resistant livestock breeds, reducing stocking density, and improved ventilation. We identified a lack of scientific literature relating to heat load in animals transported by sea and considerable potential for bias in the literature that was found. We identified the following priority research areas: (i) experimental manipulation of variables thought to influence the incidence and severity of harmful heat load, including sheep density; (ii) further assessment of the Heat Stress Risk Assessment (HSRA) model used to predict heat load events, and (iii) development of a suite of animal welfare indicators that may allow identification of 'at risk' sheep before they reach debilitating heat load condition. Addressing these knowledge gaps will assist efforts to reduce the frequency and intensity of harmful heat load events.

Keywords: cattle; heat stress; mortality; physiology; sheep; sea transport; stress; welfare

1. Introduction

Few animal welfare issues have been as persistently contentious in Australia as the 'live export' of livestock via sea [1–3]. Recent media attention [4] has highlighted concerns regarding the effects of high environmental temperature and humidity on the welfare and mortality of animals being shipped live from Australia to the Middle East, especially during the Northern Hemisphere summer. These events have affected both sheep (*Ovis aries*) and cattle (*Bos taurus* and *B. indicus*), the species most commonly exported by Australia. Given the repeated occurrence of high heat load events with elevated sheep mortalities, and less common events involving cattle [5], how the industry is regulated [6–8] has been questioned [9–11]. A lack of understanding of what variables influence the likelihood of animals experiencing harmful heat load may have hindered attempts to predict and mitigate heat load events. Narrative reviews have been published on this issue in 2014 [11] and 2016 [12], and several unpublished reviews [13,14] have been produced since a media exposé in 2018 [2,4], but to our knowledge, no systematic literature reviews have previously been performed on this topic. Systematic reviews are particularly valuable in contentious situations in which bias on the part of authors may influence which studies are included and excluded (see Supplementary Material).

Aims of This Review

This review attempts to synthesise Australian research on heat load in sheep and cattle exported by sea. To reduce author bias, we used a systematic search strategy and only included Australian studies. We attempted to synthesise what is known about this process and identify knowledge gaps where future studies are needed.

This is not a narrative review written to advance a political or ethical position. Narrative literature reviews are publications that describe and discuss the state of the science of a specific topic or theme from a theoretical and contextual point of view and the authors do not disclose how they chose to include studies. The authors aimed to report on the outcomes of published and unpublished animal-based studies without applying our own judgement as to what constitutes 'good enough' or 'unacceptable' outcomes for animals in the context of sea transport. This review does not attempt to assess heat load in contexts outside of sea transport and does not consider conditions outside of ships originating in Australia. This review does not attempt to assess animal welfare impacts of sea transport as a whole.

2. Systematic Review Methods

The authors minimised author bias by using a systematic search strategy according to the PRISMA guidelines (see Supplementary Material) to identify relevant journal articles, books, book sections, unpublished reports, conference proceedings, procedural documents and theses.

Summary of Literature Reviewed

We found a total of 93 literature items matching our criteria. These comprised 51 peer-reviewed studies (55% of literature items), 29 unpublished reports, three book chapters, five theses, two conference papers, and three procedural documents. Most literature items found were contemporary, with 86% published since 2000. Further information is provided in Supplementary Material.

3. Background to Australian Sea Transport of Livestock

Australia has been the largest exporter of live agricultural animals worldwide in recent decades, with the industry valued at AUD$1659 million in 2016, comprising 304 cattle voyages and 36 ship voyages exporting over one million sheep and over one million cattle that year [15]. Sheep and cattle have been exported from Australia via sea transport for at least 30 years, predominantly to the Middle East and South East Asia. Most sheep exported live from Australia are sourced from Western Australia, and are sent to the Middle East, with the voyages taking an average of 21 days. Most cattle are sourced from Northern Australia, and are sent to South-East Asia, with the voyages taking an average of 5 days. Voyages occur year-round [15]. During these voyages, hot environmental conditions are often encountered particularly during the Northern Hemisphere summer (May–October) [16] and the adverse impact of heat on transported animals has been reported since 1989 [17,18].

Heat Load and Sea Transport

During the sea transport of livestock from Australia to the Middle East, wet bulb temperature (WBT; an environmental measure dependent on dry bulb temperature and humidity [19]) commonly reaches 30 °C and can reach maximums between 32 °C and 34 °C with little diurnal variation [17]. Reduced diurnal fluctuation in environmental temperature during the shipment period limits an animal's opportunity to lose heat gained during the previous day, and if heat dissipation is less than the accumulation of heat within the body, the animal accumulates a "heat load" [20,21].

Dangerous heat load may occur for transported livestock in any area experiencing high sustained environmental temperatures. This may occur in many areas of Australia, particularly Northern Australia during the Southern Hemisphere summer, and has been documented for exported animals during the Northern Hemisphere summer, at ports in the Persian Gulf [5,16,22].

4. Physiology of Heat Load

The basic physiology of thermoregulation has been covered in detail elsewhere [23]. Briefly, mammals such as sheep and cattle have complicated homeostatic systems to keep their body temperatures within a reasonably narrow range. The amount of heat produced by an individual of a given mammal species will be influenced by factors such as nutrition (amount, type, and timing of feeding), body size, breed, physiological status, and acclimatisation [20]. Metabolic heat and heat from the environment can increase body temperature but mammals are able to maintain their homeostatic body temperature over a wide range of ambient temperatures by balancing heat loss or gain, and heat production. There are also behavioural responses to increased temperatures, such as changing posture (e.g., stock stand or spread out to increase surface area for heat loss, reduce activity, and seek shade if outside) [24]. However, the accumulation of heat without dissipation may result in mortality or several debilitating physiological changes. Before such adverse animal welfare events occur, acute heat load episodes exhibit a predictable pattern of physiological stages [25].

4.1. Thermal Zones

Maunsell Australia [26] cited the thermoneutral zone (TNZ) for livestock shipping as the range of environmental temperatures at which the deep body temperature should remain constant. Within that zone, body temperature can be kept in the normal range by constant heat loss through usual sensible and insensible mechanisms. The upper limit of this zone is the upper critical temperature, and when the animal is exposed to environmental conditions above that limit, body temperature rises.

4.2. The Heat Stress Threshold (HST)

In the context of sea transport, the environmental conditions are considered to be best described by WBT. The upper critical WBT beyond which body temperature rises 0.5 °C above what it would otherwise have been is expressed by the live export industry as the heat stress threshold (HST) has been defined as the WBT when the core body temperature is 0.5 °C above what it otherwise would have been [26]. The same authors defined "mortality limit" (ML) as the ambient WBT above which the uncontrollable rise in deep body temperature leads to death of the animal. The environmental WBT at which body temperature rises has been the subject of observational studies [27,28], experimental research [20,29] and much debate [13]. The data sets for establishing these WBT thresholds are somewhat limited and they have been further extrapolated to cover a wider range of animals [26]. The debate which criticises the values used for HST may not adequately distinguish the complexities of the different thermal zones, the species, breed and individual differences in response to environmental conditions. The definition of HST and the use of this definition in the HSRA may not sufficiently account for the effects of environmental conditions, acclimatisation, and thermoregulatory responses of animals [12]. The concept of HST and the HSRA model also does not take into account the cumulative effects of heat load over time and the capacity of the animals to recover during periods of respite [14].

We are unaware of any literature examining the extent to which animals experience discomfort, "stress", or distress at different body or environmental temperatures. However, some authors have attempted to relate human perceptions to how animals may be feeling e.g., [12]. Therefore, we conclude that decisions about cut-off or threshold environmental conditions have been made on physiological grounds, with less capacity to include measurements of behaviour or affective state. Clinical observations of animals subject to high environmental heat and humidity describe elevations in body temperature, with varying increases in different tissues (peripheral, rectal, core), increased heart rate, changes in peripheral perfusion, changes in respiratory rate and character, reduction in feed consumption, often an increase in water consumption, and changes in behaviour [20,29].

5. Assessment of Heat Load on Animals

5.1. Point and Cumulative Effects

The effects of heat on animals reflect both a single extreme heat insult, and prolonged cumulative effects, that is, heat load may be imposed by exposure to a short period of extreme heat, or may be the result of prolonged exposure to hot conditions, if there is no relief or cooling [21]. Australian feedlot studies have examined the effect of prolonged or chronic (110 day) heat load on cattle [30]. Duration of stress or suffering is central to considerations of animal welfare impacts generally, and this extends to livestock transport and heat load episodes in sea transport [14].

5.2. Recovery and Respite

Australian Merino sheep can maintain body temperature within the normal range during exposure to a prolonged increase in heat (maximum temperature of 38 °C, minimum temperature of 28 °C) and to recover quickly from the negative effect of heat load within two days of conditions returning to thermoneutral conditions [31]. Stockman, et al. [32] reported that Merino wethers experienced significant physiological changes during exposure to prolonged and continuous high heat and humidity, but maintained most aspects of homeostasis despite being hyperthermic and recovered quickly when conditions returned to thermoneutral. Stockman [20] described an additional experiment which subjected sheep to hot, humid conditions without any diurnal cooling, whereby the daily mean, maximum, and minimum core body temperatures became significantly elevated.

The experimental study of Beatty, et al. [33] used a climate control room (CCR) to simulate sea transport conditions (high temperature and humidity) using small sample sizes (six animals) of *B. taurus* and *B. indicus* cattle. The results of Beatty, et al. [33] suggested that *B. taurus* cattle experience significant physiological changes during exposure to prolonged and continuous high heat and humidity, with alterations persisting for days after the heat load conditions subsided. *B. indicus* cattle were observed to experience similar but less pronounced physiological changes. The cattle feedlot industry uses heat load index, and accumulated heat load, to predict the likelihood of adverse heat events [34]. Important in these models is the period of cooling which might provide respite from excessive heat. We were unable to find any studies that empirically assessed the duration of respite periods required to protect livestock from harmful cumulative effects of repeated episodes of heat load. This knowledge gap is likely important for understanding heat load in sea transport where there is sustained exposure to hot conditions.

6. Heat Load and Mortality

Livestock mortality due to excessive heat load can occur under many conditions including in feedlots and during transport. Several investigations have been performed into causes of livestock mortality in sea transport [5,35–43] and excessive heat load has been considered an important cause, particularly in sheep [17]. There are ongoing concerns for mortalities in sheep due to excessive heat load during sea transport [11,18].

6.1. Mortality Monitoring

Mortality rates on board livestock sea transport ships of 0.1–2% have typically been reported for sheep and cattle ships [15] with occasional voyages reporting much higher rates (e.g., 28.5% for cattle on one voyage) [5,22]. The proportion of these deaths that are contributed to by heat load has not always been clearly reported, with many sheep deaths appearing to be due to a combination of heat load, salmonellosis, and inanition [12]. Some authors have suggested that reported data may be unreliable due to veterinarians and livestock officers being employees of export companies and there have been allegations of under-reporting [44]. Long-term analyses have shown that livestock mortalities have been generally decreasing since data began being reported in 1995. Recent analyses reveal that sheep deaths are increased during the Northern Hemisphere summer. Evidence from shipments from 2005 to 2014 is that mortality rates rise to approximately double when sheep are transported from Australia in winter to the Middle East in summer. For example, monthly mortality rates for shipments of wethers from Fremantle to the Middle East increase from approximately 0.5% (February) to approximately 1% (August) [15]. A five-year average of total mortality rate of sheep shipped from Australia to the Middle East shows mortality rates for sheep exported to the region are higher when sheep are loaded in May to October. While this pattern may only be an association, there is an "enduring stability of seasonal difference" of mortality rate in all classes of sheep over time [15]. Additional factors influence the seasonal effect on mortality rates such as in any given year, there is variability between ports with respect to average annual mortality rate [16].

6.2. Monitoring Outcomes other than Mortality

Mortality is one of many adverse animal welfare events whose frequency may be quantified in monitoring programs and represent the extreme end of suffering that may occur due to heat load [45,46]. For this reason, monitoring of adverse events beyond mortality has been suggested by many authors and McCarthy [14] recently recommended that the industry moves away from using mortality as a measure to a focus on measures that reflect the welfare of the animal including those that reflect heat load. Additional careful monitoring of animal behaviour at the pen-level, such as panting, eating, and resting behaviour of stock should be pursued. These data should be combined with basic environmental measures, also at the pen-level, such as temperature, relative humidity, and measures of ventilation [47].

7. Factors Affecting Heat Load In Transported Livestock

Considerable research has been devoted to investigating the influence of several controllable variables on heat load experienced by transported animals, including diet [48,49], ventilation [50], and environmental conditions [51].

7.1. Environment-Based Factors

Environmental conditions with sustained high temperatures predispose animals to harmful heat load. When hot conditions also include low diurnal temperature fluctuations, animals are further predisposed to harmful heat load. This limits an animal's opportunity to lose heat gained during the previous day and can compound the effects of subsequent heat load. Humid conditions further reduce the capacity for animals to lose gained heat. Hot, humid conditions with low diurnal fluctuation are often encountered during the voyages of livestock transport ships to the Middle East in the Northern Hemisphere summer [13].

7.2. Management-Based Factors

Altering the physical environment may reduce the heat load on animals. For instance, provision of shade will reduce heat gain from solar radiant heat gain; in shipping this is only of relevance where there are open decks and animals may be exposed to direct sunlight. Radiant heat gain from hot metal infrastructure may maintain temperatures so there is little respite for animals housed within; there are currently limited options for cooling the ships other than wetting with cooler water [52].

7.2.1. Stocking Density

Stocking density or space allowance is an important factor underlying heat load, and of prominent concern for livestock welfare. Incoming air from mechanical ventilation accumulates additional heat and moisture from the animals, generated through their metabolic processes. The HSRA uses in its calculations a "wet bulb rise" which describes mathematically the contribution of animal metabolic heat to the environmental conditions experienced by the animals in the ship [53]. Therefore, the stocking density of animals (head per square meter) has a strong influence on the heat load experienced by animals. Aspects such as the underlying metabolic rate and body size of animals will affect how much heat they release and metabolic rate is also affected by how much they eat. The capacity of the ventilation system to remove waste gases, such as carbon dioxide and ammonia, may further influence stocking density for each deck and area [53,54]. The expiration of carbon dioxide is dependent on the animal's metabolic rate and the substrate metabolised, such that higher stocking density of animals may generate more carbon dioxide than can be adequately removed from an area. Ammonia in shipping is primarily generated through chemical breakdown of urea in urine and faeces (and bedding) and therefore may reach greater concentrations with higher animal density and increased waste production [55].

7.2.2. Ventilation

The role of ventilation in sea transport has been investigated by industry-funded research projects [50,53,56]. Livestock transport ships may either have open decks (natural ventilation) or closed decks with mechanical ventilation [53]. Most livestock vessels rely on mechanical ventilation, which serves three main purposes. Firstly, it replenishes air (including oxygen). Second, it removes heat. Third, it removes waste gases, including water vapour (evaporates moisture from the manure pad), carbon dioxide, and ammonia [14]. Ammonia is a highly irritating alkaline gas that has been associated with adverse effects on sheep on transport vessels [57,58]. Air movement is considered to be important and airspeed can be used to give an 'adjusted WBT' [53]. On ships equipped with forced ventilation systems, pen air turnover (PAT) and speed of air flow are two aspects of air movement which are considered within management models regarding carriage of livestock [27,53,56]. The mechanical ventilation systems currently used on livestock vessels work on high air turnovers (e.g., 50 m/h or m^3/h divided by m^2) which are required to remove gases and assist in removing moisture from faecal pads [53]. Increased flow of cooler, drier air will enhance convective and evaporative heat loss. If the air is hotter than the animals, or saturated with moisture, the cooling effect is diminished, and hot, humid air may contribute to heat gain rather than heat loss.

It has been proposed that a risk management approach may be required for operations involving open deck pens with no mechanical ventilation [53]. Ships require mechanical ventilation of open decks only when the breadth is greater than 20 m, otherwise they rely on natural ventilation. McCarthy [14] has recommended that all vessels should be re-certified to determine pen air turnover, air speed, and ventilation patterns, before travelling to the Middle East during the Northern Hemisphere summer.

7.2.3. Provision of Feed and Water

Feed intake can be influenced by heat load [59] and feed type can influence heat production. Nutritional management of animals in hot conditions can involve reducing total energy input, through provision of feeds with higher roughage content or by restricting total feed intake [60]. It is common management practice to introduce feed restrictions in high-risk heat load conditions in order to limit excessive heat output from animals due to digestion [61]. Time of feeding can also altered to coincide with the cooler part of the day. Kennedy [61] performed a CCR experiment that investigated the effect of different grain feeding approaches on cattle (16 steers) subjected to heat load over three days. He reported that when environmental heat load was imposed in a CCR, cattle fed a wheat diet showed greater thermal stress than cattle fed a sorghum diet, but when animals were subjected to a second period of heat load, the result was equivocal [61].

Provision of supplements may assist animals in responding to the heat [62,63]. It has been suggested that employing a dietary supplement may be a cost-effective and simple method for ameliorating the negative impact of heat load in sheep [48]. Electrolyte supplementation of cattle under hot conditions is proposed to assist with the acid-base changes that occur due to panting. Beatty, et al. [49] provided electrolytes in feed and water to 80 *B. taurus* steers on a livestock transport ship and reported higher live weights in the supplemented cattle. While some degree of heat load was observed during the trial, the steers were not considered clinically heat stressed during the experiment. It was not apparent from CCR research performed with sheep that electrolyte supplementation was similarly beneficial [20], although there may be acid-base and electrolyte changes in extreme conditions.

Supplementation with supra-nutritional doses (beyond required levels) of Vitamin E and selenium may ameliorate effects of high heat load in sheep [64–68]. Betaine (trimethylglycine), an amino acid capable of acting as an organic osmolyte or a methyl donor, can improve animal production measures in cattle, pigs, poultry and lambs and has been suggested as a useful supplement for heat load management in sheep [48]. Drinking water temperature may affect heat load in livestock. Offering chilled water to sheep [69] may be a useful method to decrease body temperature during times of high heat load, although it has been shown that sheep and cattle will drink greater volumes of warm water [69]. Further research is required to determine if electrolyte supplementation for cattle and nutritional supplementation for sheep would be beneficial and feasible for on-ship use when animals endure periods of high head load.

7.2.4. Management of Pens Including Bedding and Manure Pad

Provision of bedding is linked to ventilation and air quality [70] and may influence heat load experienced by transported livestock. In Banney, et al. [71] the link between air quality and bedding is described, whereby ventilation will affect the moisture content of the bedding, and the removal of noxious gases produced in the bedding. It is important that the bedding does not contribute to further production of heat or noxious gases, such as ammonia or carbon dioxide, as might occur when organic matter ferments. A variety of materials have been tested or used for bedding in animal industries, including sawdust, straw, woodchips, pine shavings, and desiccated manure [70]. Sawdust is the most frequently used material for cattle and is required on 'long-haul' (>10 day) voyages.

The manure pad from sheep is generally quite dry, and if it remains firm, dry and intact, it is considered by the industry to be the preferred choice of bedding material for sheep during sea transport [1,55,71]. However, if the sheep manure pad becomes excessively wet, it can contribute to problems with the production of noxious gases [55], with sheep having difficulty moving around ("pugging"), and with faecal contamination of the legs and body of the sheep. When there is high environmental heat, with increased humidity and increased urine output from sheep drinking more, the ventilation may not be able to keep the manure pad sufficiently dry, exacerbating the problems [71]. The manure from cattle being more liquid than that from sheep means generally the cattle pens need more regular cleaning during long haul voyages, although this might not be necessary during short haul voyages. Banney, et al. [71] describe in detail the processes around washing down the cattle pens, and the cattle themselves, with the addition of new sawdust after the washing. They note the advantages of washing and wetting the cattle in providing some cooling relief during very hot conditions, but underline the essential role of good ventilation at that time in limiting a rise in humidity.

7.3. Animal-Factors

7.3.1. Breed Selection

Animal factors can be manipulated to ameliorate the adverse effects of heat load on livestock. Specifically, selection of breeds arising from hot regions over those evolved in temperate regions generally improves tolerance to heat load. For instance, *B. indicus* cattle (originating from South Asia) generally have greater heat tolerance than *B. taurus* cattle (originating from Europe) [46,72–76]. Omani, Niamey or Awassi sheep breeds (Middle Eastern) similarly have greater heat tolerance than Australian Merino sheep (originally from Europe) [20,77]. While *B. indicus* cattle are abundant in Northern Australia [46], the availability of Middle Eastern sheep breeds in Southern Australia, when compared to Merino flocks, is much lower. As such, changing sheep breeds in sea transport could only be a long-term strategy that would require economic modelling as it would impact on farm profitability.

7.3.2. Acclimatisation

Acclimatisation of animals to heat requires exposure to hot conditions for several days or weeks [76,78]. With respect to live export, this means acclimatisation to heat or cold should be in place before the transport process commences ideally, so that the animals are prepared in a climate similar to which they are travelling. During that time, there will be behavioural and physiological responses that decrease metabolic heat production, such as decreased feed intake and metabolic rate, and other responses that improve their ability to lose heat, such as increased sweating, and higher plasma volume [29].

7.3.3. Effect of Fleece on Sheep

Experimentally, shearing has been shown to significantly increase the heat tolerance of rams, presumably by enhancing the efficiency of evaporative cooling from the skin [79]. Anecdotal reports have suggested that recently shorn sheep cope better than fleeced sheep with hot conditions encountered during the sea transport voyages to the Middle East. As a consequence, sheep destined for sea transport may be shorn in the immediate period before shipping, to limit wool cover and so improve heat loss [80]. Beatty, et al. [81] tested this hypothesis with a CCR experiment involving shorn and fleeced Merino sheep. They found that fleeced sheep maintained higher core and rumen temperatures and respiratory rates than shorn sheep under all environmental conditions. Maunsell Australia [50] reported that when WBT was >26 °C on livestock transport ships, unshorn ewes were hotter than shorn ewes by 0.2 °C to 0.4 °C as measured by rectal temperature.

There are concerns that pre-embarkation shearing may contribute to increased stress, and inappetence. To address these concerns, an experiment was performed whereby 600 sheep were fitted with Radio Frequency Identification tags, and subsets were shorn each day (days 1, 2, 3, 4 or 5) and time and frequency of feed and water trough attendance were determined [80,82]. There was no difference in time spent at feed or water troughs between any treatment groups on any day, and minimal behavioural changes were observed. This suggests that shearing may occur on any day during the pre-embarkation feedlot period, and that current management practices regarding shearing do not disrupt time spent feeding.

8. Pathways for Reducing Excessive Heat Load in Livestock Sea Transport

Risk assessment approaches have been developed and refined by the sea transport industry for anticipating conditions likely to precipitate heat load episodes [5,51,83]. The response variable traditionally underlying this approach has been animal mortality but it has recently been proposed to replace the mortality limit with a heat tolerance level within the risk assessment model [14]. Risk assessment approaches have also been developed for heat load management in feedlot cattle [84]. Critics of the Australian government's risk assessment approach have argued that the estimate of the heat stress threshold of sheep used in the model is substantially higher than that observed under simulated sea transport conditions, which may lead to an underestimate of the importance of heat load in sheep on voyages where mortality is high [12]. It is widely recognised that further improvements are required to reduce the incidence of harmful heat load episodes for exported sheep [14]. Suggested pathways for reducing the incidence and severity of harmful heat load episodes for exported livestock are listed below from most drastic to most subtle.

8.1. Avoidance of Seasons and Extreme Weather Events

Proposals have been made that sea voyages should be avoided in the Northern Hemisphere summer. Scientific reviews have suggested that there is elevated risk to sheep exported from Australia during summer in the Middle East and ethical arguments have been advanced that this risk is sufficient to warrant consideration of restriction of trade during this period [12,13].

Heat Load Forecasting

Considerable research has been devoted to forecasting heat load for animals in feedlots [84–86]. Heat load forecasting within the Australian feedlot industry has evolved over a period of two decades [87]. These developments have been used by the sea transport industry to generate a computer model that aims to assess the risk of heat stress and to contain mortality levels on livestock ships below certain arbitrary limits. The Heat Stress Risk Assessment (HSRA) model 'HotStuff' model was developed for the Australian livestock export industry to estimate and minimise the incidence of heat stress mortality in livestock during voyages to the Middle East [26]. The model has been in operation since 2003 and has had several refinements and reviews [51,83]. The HSRA model provides a framework from which to address heat stress and heat load. The model factors in the weather (both predicted and actual for the destination ports), the type, class and body weight of animals, and ship factors such as ventilation design and airflow. The latter requires input from the vessels' design specifications and is adjusted for each vessel prior to loading [26].

However, despite the sophistication of the model, it often fails to accurately predict a heat load event [87]. The accuracy of the HRSA model is currently being questioned, and the ability to prevent animals experiencing harmful heat load when travelling to the Middle East may be limited by the ventilation capacity of the vessels. Given mounting recent evidence of ongoing heat load events affecting exported sheep, it is argued that a review of the settings used for the input of data in addition to a review of the risk settings should be undertaken [14]. The current settings used is a 2% probability of a 5% mortality due to heat stress, and was chosen by industry [51,83]. It has recently been suggested that the risk setting should be replaced by the likelihood of an animal experiencing heat stress, not mortality, in order to achieve improved welfare outcomes [14].

It has also been suggested that revision of the HSRA model should include consideration of the different heat load thresholds, with examination of the appropriateness of using 0.5°C rise in body temperature, the WBT at which that occurs, and the duration of that episode, as an arbitrary threshold [13]. Phillips [12] criticised the HST values used within the HSRA model as being above those suggested by animal studies. It is important to note that some of the discussion in recent reviews regarding heat stress thresholds (HSTs) do not appear to fully appreciate the distinctions between how different researchers have expressed the thresholds. For example, Stockman [20] described three different HSTs. The HSRA further iterates ML for different classes of stock, from a base animal of specified type, weight, and normal body temperature. Ferguson, et al. [51] did not criticise the methodology or base values in their review of the model.

The HSRA model does not yet have the capacity to deal with the effects of cumulative heat load. It would be advisable for future revisions of the model to use expertise from the feedlot industry to consider the influence of duration of heat exposure and the capacity for respite to influence effects of heat load.

8.2. Improved Ventilation

The issue of ventilation is central to heat load events in sea transport. There are several pathways that have been proposed for refinement of current conditions. Ships with 'open decks' (lacking mechanical ventilation) may pose extra challenges for improving air flow during high-risk heat load conditions [50,53,56]. Simple maintenance steps have been proposed to ensure that existing ventilation systems are operating at maximum capacity. First, removal of anything that obstructs airflow in and around the decks can be performed. Second, regular checking of fans to ensure they are working at full capacity can be performed. Third, it is important to ensure that all exhaust outlets are free of obstruction [14].

Air Conditioning

The approach of installing air conditioning in livestock transport ships has been considered as an avenue for cooling animals during high-risk conditions. However, air conditioning requires a low air turnover (and often recycling of air) in order to be effective (and/or cost-effective). Low air turnover systems would likely be less effective at removing waste gases and faecal moisture from animal pens, and for this reason, with current available technology, air conditioning does not seem to be a viable option for livestock vessels [14].

8.3. Reducing Animal Density

It has been argued that currently used stocking densities on sheep voyages are too high [13,14], contributing to too great a WBT rise, especially when the prevailing environmental conditions are already close to the HST. The HSRA model uses stocking density as a critical factor in determining WBT rises across the deck. As detailed above, there is little scientific literature to allow accurate elucidation of threshold densities that would safeguard cattle and sheep across all voyages, and the HSRA has extrapolated data for risk management. However, given that stocking density is the key parameter in managing livestock in long haul voyages to the Middle East, sizable reductions in allowable stocking densities have recently been suggested [13,14]. Further studies should be undertaken to examine the effects of such changes across stock classes. The use of allometric equations [88,89] to determine space allocation of livestock undergoing sea transport has been proposed, and further work is required to determine the appropriate *k* values for use in allometric equations to appropriately limit the wet bulb rise. Reducing stocking density may also improve other animal welfare issues such as the ability of animals to cope with ship motion [90].

8.4. Improving Bedding

Exposure to high environmental heat causes animals to drink and urinate more [32,33], which then increases moisture in the pens. Excess moisture in the sheep faecal pad can be managed by providing additional sawdust, a useful interim measure to ensure that sheep don't get bogged down or have fleece covered with faecal material [70,71]. Exact ventilation rates to ensure adequately dry faecal pads have not been determined given these will vary with climate, pen location, ship type and sheep density. The careful management of hosing in cattle pens has also been suggested to improve bedding during times of high heat load [52].

8.5. Wetting

Wetting of animals can be used to mitigate high heat loads via lowering the temperature of the microclimate surrounding animals. This approach has been used for cattle in feedlots and on livestock ships [52]. Wetting has been trialed and has been shown to lower peak body temperatures and hasten recovery from hyperthermia. Four water application methods (hosing, overhead sprinklers, leg sprinklers, and misters) were evaluated by Tait [52] in a CCR experiment with *B. taurus* cattle, and high-pressure hosing was found to be the most effective. It is unknown how practical water spray cooling would be on-board ships or how effective it would be for sheep. There are several concerns about the use of wetting for sheep. Wetting has the effect of increasing local humidity, causing undesirable wetting of the faecal pad and wetting the fleece of sheep which will only slowly dry in humid conditions. For these reasons, wetting has not been further explored for preventing harmful heat load in transported sheep but is considered to be a useful approach for cattle [52].

9. Conclusions

Commercial transport of livestock by sea from Australia has been conducted for over 30 years, and although there has been continual improvement in mortality incidence [15,91], high mortality events associated with heat load continue to occur. Moreover, public concern for the welfare of transported animals is elevated since a 2018 media exposé on the negative effects of heat load [2]. Despite the imperative for objective understanding of this condition, we found some potential for author bias in the literature. There has been insufficient independent science (55% of literature items we found were peer-reviewed) which addressed heat load and sea transport of livestock, with much of the literature consisting of non-peer-reviewed reports funded by industry and narrative reviews written to express opposition to the industry. Animal-based studies indicate that harmful heat load is often observed in livestock transported by sea from Australia, particularly in sheep and cattle sent to the Middle East in the Northern Hemisphere summer. For the industry to be socially sustainable, further scientific investigation is required to identify avenues for reducing the incidence of harmful heat load events. The extent to which avoiding consignments in the hot summer months may be sufficient to avoid the majority of incidents of heat stress remains untested.

Trade in live animals can bring substantial financial benefits for exporters and importers [92], but it requires the trust and goodwill on the part of both trading partners as well as community support in democratic countries. Improved understanding of the effects of heat load on livestock, when extreme heat load occurs, and ways to prevent its occurrence are required to minimise animal welfare impacts for livestock transported by sea. Eliminating the risk of extreme heat load would likely require reductions in stocking densities, improvements to ship ventilation and on-board stock management, and possibly ceasing shipments to the Middle East in the Northern Hemisphere summer. These factors combined may mean that some transport consignments become uneconomic, highlighting the difficulty of balancing animal welfare and economics in this context. In states like Western Australia, where the sheep industry is underpinned by live export, such trade interruptions could have deleterious impacts on industry viability.

Prudent suggestions from what is known include moving away from using mortality as the main determinant of animal welfare outcomes [14] and development of multiple animal-based parameters. Ongoing adverse heat load events suggest that further review of the currently used HRSA is vital. Studies that determine the duration of respite periods required to protect animals from harmful cumulative effects of heat load are essential. In addition, studies that can describe and validate a list of welfare indicators that reflect the physical and affective state of animals should be considered [93]. In the first instance, careful monitoring of animal behaviour at the pen-level, such as panting, eating and resting behaviour of stock should be pursued, combined with basic environmental measures, such as temperature, relative humidity, and measures of ventilation [47].

Concurrently, studies in land-based facilities that can examine direct effects of changing one variable at a time (e.g., varying stocking density in CCR experiments) will be informative. Given this type of research will take time for relationships between factors to be described, interim measures could be commenced immediately to reduce the short-term incidence of harmful heat load from existing knowledge. Such measures may include reducing stocking density, for instance using allometric principles, providing adequate bedding to ensure a consistently firm faecal pad, and providing more detailed monitoring of livestock. There are likely to be important interactions between ship factors (ventilation), management (stocking rate, fodder, bedding), and animal (body size, weight, breed) factors. The importance of each of these will vary according to voyage length and climate. Unless pathways can be found to reduce the incidence and severity of heat load episodes, and to demonstrate these improvements in refinement of animal welfare outcomes, the community support and sustainability of this form of livestock transport may well expire in Australia.

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animals MDPI

Article

Road Transport of Farm Animals: Mortality, Morbidity, Species and Country of Origin at a Southern Italian Control Post

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Simple Summary: Long distance transportation is a welfare concern because it may cause sickness (i.e., morbidity) or death (i.e., mortality). Commercial transportation in Europe is regulated by the Council Regulation (EC) No. 1/2005 which regulates the maximum journey in the different species. After this time animals must be unloaded for resting, watering and feeding at control posts (CPs) where Official Veterinarians (OVs) have to check their health. This study analyzed the surveillance reports filled by OVs at a CP in Southern Italy from 2010 to 2015. A total of 1391 trucks stopped at the CP, transporting a total of 111,536 animals. The average mortality and morbidity rates were 0.025% and 0.010%. Cases of mortality and/or morbidity were reported for only 11 out of the 1391 trucks (0.8%). In a truck transporting lambs, 14 dead on arrival (DOA) were recorded, and this represented 93% of all DOAs. This is the first study reporting the results of surveillance practices conducted by OVs on animals travelling from North Europe to a CP in Southern Italy in compliance with EC 1/2005. Further studies should be conducted comparing the implications of long distance transportation at different CPs along different routes.

Abstract: Statistics on animal transport and its implications for health and welfare are limited. This study documented the animals transiting through a control post and their welfare outcomes measured by mortality rate and the prevalence of animals considered unfit for further transport (i.e., morbidity). Reports filed by the director of the control post and Official Veterinarians from 2010 to 2015 were analyzed. A total of 60,454 (54.2%) sheep/goats, 45,749 (41.0%) cattle, and 5333 (4.8%) pigs travelled in 225 (16.2%), 1116 (80.2%) and 50 (3.6%) trucks, respectively. Trucks coming mainly from France (71.3%), Spain (14.0%), and Ireland (7.4%) went mainly to Greece (95.4%), which was also the most common nationality of the transport companies (44.6%). Cases of mortality and/or morbidity were reported for only 11 out of the 1391 trucks (0.8%). The average mortality and morbidity rates were 0.025% and 0.010%, with maximum values for transport of lambs (0.084%, and 0.019%). Species of animal being transported and space allowance were associated with the measured welfare outcomes ($p < 0.05$). Overall, this study provided statistics based on official surveillance reports, suggesting that small space allowance during long haul transportation of sheep/goats may affect their health and welfare.

Keywords: livestock; transport; control post; health; welfare

1. Introduction

Millions of animals are transported daily all over the world. The movement of livestock across the borders of Member States of the European Union is monitored using the Trade Control and Expert System (TRACES) and reported in the Activity Report [1]. For example, approximately 3 million head of cattle are transported for fattening annually. However, long distance animal transport is an animal welfare issue, because it is a stressfull event triggering often the onset of health problems [2]. With the aim of reducing transport stress and consequently the incidence of transport-related health and welfare issues, many studies have been published identifying risk factors for farm animals pre-, during and post-road transport [3]. Pre-journey risk factors include many factors, such as on-farm handling, rearing conditions, assembly of animals, classifying, weighing, repenning in a new environment, re-grouping, mixing with unfamiliar animals, fitness for transport and handling at loading [4,5]. Among risk factors during the journey are journey duration, withdrawal of feed and water, thermal and physical conditions inside the vehicle, overcrowding, absence of partitions, driving skills, noise, vibration, and road quality [6–8]. Post-journey risk factors include handling at unloading, duration of rest period, recovery practices, re-grouping, and mixing with unfamiliar animals [9–12].

One of the determining risk factors is journey duration [13,14]. Consequently, Council Regulation (EC) No. 1/2005, which regulates animal transport in Europe, includes special requirements for journeys exceeding 8 h. For instance, maximum journey duration is 29 h for ruminants and 24 h for horses and pigs. After this time animals must be unloaded for resting, watering and feeding for at least 24 h in locations approved by the competent authorities [15]. Such locations used to be called staging points in Council Regulation (EC) No. 1255/1997 and have now been renamed control posts (CPs) by Council Regulation (EC) No. 1/2005. Council Regulation (EC) No. 1255/1997 (Article 6) requires that official veterinarians (OVs) inspect the means of transport and accompanying documents, as well as evaluate the animals' fitness for transport before the animals leave the control post again. The facilities and management at CPs have been identified as key factors in animal recovery, affecting both resting behaviour and biochemical parameters [10,11,15]. However, scientific literature regarding the effect of CPs on animal welfare during long distance road transports is still limited.

Surveys on farm animal transport have been performed to explore the epidemiological basis of transport-related health and welfare issues worldwide. For instance, the mortality due to road transport has been calculated for beef cattle in North America (0.01%) [16], fattening pigs in Europe (0.07%) [17], and bobby calves in Australia (0.64%) [13].Whilst death is a definitive welfare outcome, the variation in the above mentioned mortality is most likely related to the species or the type of animals being transported and their transport and handling conditions [18], or else to the journey duration [14]. The prevalence of transport-related health problems varied significantly even within the same species (e.g., in slaughter horses injury rate varied from 7% to 28% [19,20]). One reason for this large variation may be the use of different criteria to assess health problems. To the best of the authors' knowledge, there is no survey reporting animal transport welfare outcomes measured by OVs at a control post. Italy currently has 13 CPs, only one of which is in Southern Italy (Doc. SANCO/2677/99 Rev.241). Consequently, the aims of this study were to document animal transits through the Southern Italian control post from 2010 to 2015 and to report the corresponding mortality and prevalence of animals considered unfit for transport using the official reports filed in compliance with Article 6 of Council Regulation (EC) No. 1255/1997 and Annexes I and II of Council Regulation (EC) No. 1/2005.

2. Materials and Methods

2.1. Dataset

With the permission of the OVs and the director of the control post, surveillance reports from 2010 to 2015 filed by the director of the control post (CP) IT CE 07/PS Bitritto (Bari, Italy) in compliance with Council Regulation (EC) No. 1255/1997 (Article 5(h)), and double-checked by OVs from the Local Health Authority (Bari, Italy) were used for this manuscript.

The CP (authorization CE 07/PS; 41°2′27″ N, 16°50′9″ E) measured approximately 8000 square metres during the data collection period and could contain up to 1200 bovines, 1850 sheep/goats, 500 pigs and 150 horses. It had a lorry wash, three hay barns, five animal houses and many different pens, equipped with watering and feeding points, bedding and resting facilities. The animal houses had adequate natural and artificial light to ensure proper inspection of the animals by the OVs.

At the control post, three OVs working for the Local Health Authority (Bari, Italy), including one of the authors (DT) checked the animals during loading, and immediately before they left the CP, to assess their fitness for transport following the criteria set out in Annex 1 of Council Regulation (EC) No. 1/2005. Depending on the day of arrival, the animals were checked by one of the three OVs. However, those three OVs had each received official EU training to assess fitness for transport and used the same check list to compile the surveillance reports. In compliance with the (EC) No. 1/2005, the OVs judged animals as unfit to continue the journey when they were either seriously injured or presented clinical signs of a pathological process, including severe lameness (animals unable to move independently without pain or to walk unassisted), prolapse, severe open wound, and respiratory and gastro-enteric disease (animals with massive nasal discharge, severe dyspnoea and pleurodynia, animals with severe diarrhoea or acute abdominal pain, respectively). Such animals were either humanely euthanized, slaughtered in the local slaughterhouse or treated depending on the severity of the case after the assessment performed by the OVs.

The following parameters were recorded in the surveillance reports: date and time of arrival, species, number of animals transported per truck (NATT), country of provenance, TRACES code, truck registration number, transport company, number of dead on arrival (DOA), of those which died at the control post (DCP), of animals judged unfit to continue travel (UFT), day and time of departure, and country of final destination. Since these reports were official documents, there were no missing data.

Based on the surveillance reports, the dates were expressed as month and season. Using the category which was reported in each TRACES, the category of transported animals was added to the dataset. The categories were chosen in compliance with Council Regulation (EC) No. 1/2005, for bovines and sheep/goats the following categories were used: small calves (100 kg), medium-sized calves (200 kg), heavy calves (325 kg), heavy cattle (550 kg), very heavy cattle (>700 kg), lambs, and sheep/goats; whereas for pigs, the following categories were applied: light fattening (110–120 kg), heavy fattening (130–150 kg), and breeding (>200 kg).

The space allowance per animal was also added to the dataset. It was calculated by dividing the available floor space in each truck by the number of animals transported in the truck and expressed as m^2/animal. It was not possible to calculate the space allowance in kg/m^2 because the surveillance reports did not report the total weight of the truck load.

The transport companies were aggregated by country of origin (i.e., nationality) and the total number of dead animals was calculated as the sum of DOA and DCP.

2.2. Statistical Analysis

Descriptive statistics of the data collected were obtained using an online statistical software (Statulatorbeta®, Sydney, Australia) with year, season, month, species, category, country of provenance, nationality of transport company and country of destination as categorical variables, while NATT, DOA, DCP, total dead, UFT and space allowance were used as numerical variables.

Chi-square tests were conducted to determine the association between factors (i.e., year, season, month, species, typology, provenance, destination). Trucks with DOA, DCP, and/or UFT were considered as having a welfare problem. A univariate logistic regression model was developed with welfare problem as a binary outcome (1/0; welfare problem/non-welfare problem) and year, season, month, species, category, provenance, destination, nationality, and space allowance as predictive variables. *P* values were calculated using the Wald test. Each predictor variable returning a $p < 0.25$ from the univariate analyses was considered for inclusion in the final multivariate model for welfare problems. Predictor variables for the final multivariate logistic regression model were selected using a step-wise backward elimination procedure, whereby predictive variables were removed until all variables in the final model had a $p < 0.05$ indicating significance [9]. The aforementioned statistical analyses were performed using GenStat® Version 14 (VSN International, Hemel Hempstead, UK).

The effect of year, season, month, provenance, destination, transport company nationality, species and category on NATT was determined using a General Linear Model (GLM) procedure. Tukey's HSD (honestly significant difference) test was used as a post-hoc test. Statistical analyses were performed using SAS version 9.4. P threshold was set at 0.05. Data are expressed as least square means ± standard error (SE).

3. Results

A total of 1391 trucks, mainly trailers and semi-trailers, stopped at the control post over the study period, transporting a total of 111,536 animals. Figure 1 shows the descriptive statistics of the categorical variables year, month, season and species.

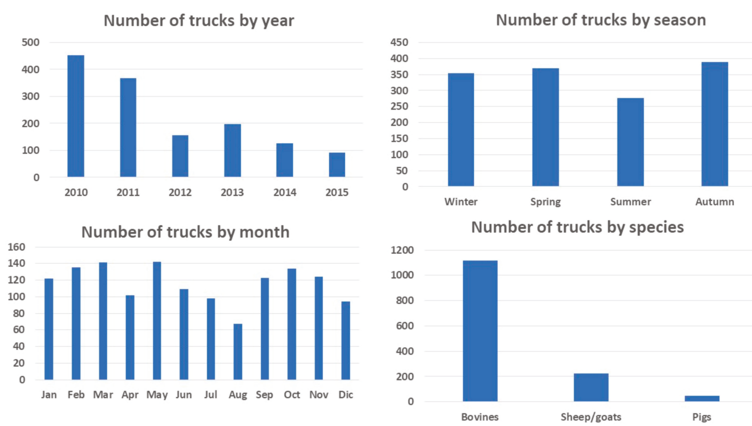

Figure 1. Descriptive statistical analysis of the 1391 trucks which stopped at control post CE 07/PS Bitritto (Bari, Italy) from 2010 to 2015. Number of trucks examined by categorical variables (i.e., factors) year, season, month, and species.

The frequency and the percentage of all the trucks transiting across the control post based on category, provenance, nationality of transport company and destination are shown in Table 1 and Figure 2, respectively.

Table 1. Frequency table by category of animal transported for the 1391 trucks which stopped at control post CE 07/PS Bitritto (Bari, Italy) from 2010 to 2015.

Species	Category	Frequency	Percentage
Sheep/goats	Sheep/goats	161	11.6
	Lambs	64	4.6
	Total	225	16.2
Bovines	Medium size calves (100 kg)	136	9.8
	Heavy calves (200 kg)	165	11.9
	Medium size cattle (325 kg)	96	6.9
	Heavy cattle (550 kg)	708	50.9
	Very heavy cattle (>700 kg)	11	0.8
	Total	1116	80.2
Pigs	Light fattening (100–120 kg)	40	2.8
	Heavy fattening (130–150 kg)	5	0.4
	Breeding	5	0.4
	Total	50	3.6
Total		1391	100

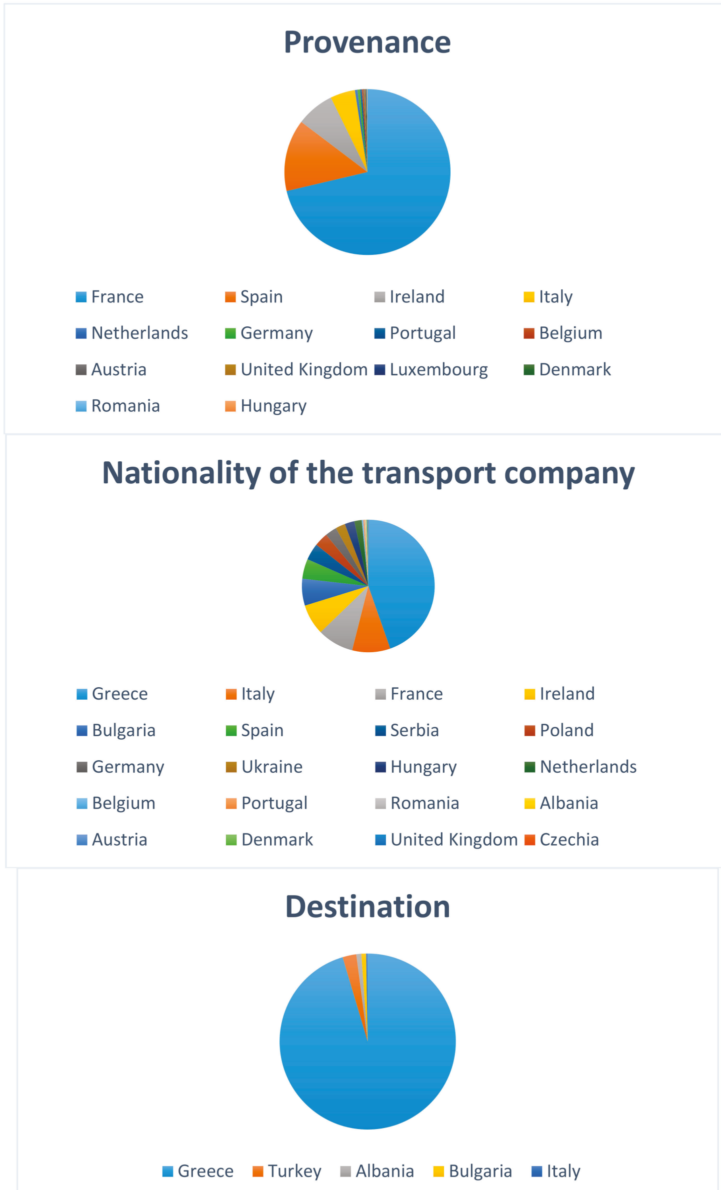

Figure 2. Pie chart by provenance, nationality of transport company, and destination for the 1391 trucks which stopped at control post CE 07/PS Bitritto (Bari, Italy) from 2010 to 2015.

The majority of the trucks transported bovines, while heavy cattle (i.e., animals with a body weight of about 550 kg) was the category most frequently transported. The most common provenances were France, Spain, and Ireland, even though the transport companies were mainly from Greece, which was also the most common final destination. There was a decrease in the number of trucks and transported animals after 2012, in particular for bovines and sheep. The transport of bovines tended to drop in August, while the transport of sheep/goats peaked in July and October (Figure S1).

Table 2 shows the descriptive statistics of the numerical variables based on the total shipments. The maximum number of DOAs (14) was recorded in a truck transporting a total of 214 lambs from Spain, and this represented 93% of all DOAs.

Table 2. Descriptive statistics of the numerical variables for the 1391 trucks which stopped at control post CE 07/PS Bitritto (Bari, Italy) from 2010 to 2015.

Variable	Mean	SD	Q1	Median	Q3	Minimum	Maximum	Sum of Values
NATT	80.18	90.20	32	37	65	9	505	111536
DOA	0.01	0.38	0	0	0	0	14	15
DCP	0.01	0.15	0	0	0	0	4	13
Total dead	0.02	0.40	0	0	0	0	14	28
UFT	0.01	0.15	0	0	0	0	4	12
Space allowance	1.58	0.75	1.06	1.84	2.13	0.21	7.56	.

Legend. SD: Standard Deviation; Q1: first quartile; Q3: third quartile; NATT: number of animals transported per truck; DOA: Dead on Arrival; DCP: Dead at the Control Post; UFT: Unfit for Continue to Travel.

Space allowance varied accordingly with the species, and was within the range reported in CE 1/2005, which regulates a space allowance from 0.30 to 1.60 m^2/animal for bovines, from 0.20 to 0.50 m^2/animal for sheep and at least 235 kg/m^2 for pigs of 100 kg. In our study, the minimal values of space allowance resulted in lamb transportation (Median = 0.34, IQR: 0.27–0.34).

The mortality and morbidity rates were 0.025% and 0.010%, respectively, with maximum values for transport of lambs (0.084%, and 0.019%). Cases of mortality and/or morbidity were reported for only 11 out of the 1391 trucks (0.8%). Table 3 shows the number of transported animals, mortality and morbidity rates calculated in relation to the different factors studied.

Table 3. Frequency table with overall calculated mortality and morbidity rates and calculated mortality and morbidity rates per year, month, season, species, and category. Total dead = dead on arrival (DOA) + dead at the control post (DCP).

Factor	Animals (n)	Total Dead (n)	Mortality Rate (%)	UFT (n)	Morbidity Rate (%)
Year					
2010	34,032	4	0.011%	4	0.011%
2011	30,171	5	0.016%	5	0.016%
2012	12,151	16	0.131%	1	0.008%
2013	16,427	2	0.012%	0	0
2014	9146	0	0.000%	1	0.010%
2015	9609	1	0.010%	1	0.010%
Month					
January	8339	1	0.011%	0	0
February	8685	4	0.046%	4	0.045%
March	8064	0	0.000%	0	0
April	8240	1	0.012%	1	0.012%
May	8205	0	0.000%	1	0.012%
June	9421	0	0.000%	0	0
July	10,013	2	0.019%	1	0.009%
August	6598	1	0.015%	1	0.015%
September	11,040	14	0.126%	0	0
October	13,776	4	0.029%	4	0.029%
November	11,413	1	0.008%	0	0
December	7742	0	0.000%	0	0

Table 3. *Cont.*

Factor	Animals (n)	Total Dead (n)	Mortality Rate (%)	UFT (n)	Morbidity Rate (%)
Season					
Winter	23,169	5	0.021%	4	0.017%
Spring	26,117	1	0.003%	2	0.007%
Summer	26,697	3	0.011%	2	0.007%
Autumn	35,553	19	0.053%	4	0.011%
Species					
Pigs	5333	0	0.000%	0	0
Sheep/goats	60,454	23	0.038%	9	0.014%
Bovines	45,749	5	0.010%	3	0.006%
Category					
Sheep/goats	39,028	5	0.012%	5	0.012%
Lambs	21,426	18	0.084%	4	0.019%
Medium size calves	9177	1	0.011%	1	0.011%
Heavy calves	9136	4	0.044%	1	0.011%
Medium size cattle	4188	0	0.000%	0	0%
Heavy cattle	23,009	0	0.000%	1	0.004%
Very heavy cattle	239	0	0.000%	0	0%
Light fattening	4758	0	0.000%	0	0%
Heavy fattening	350	0	0.000%	0	0%
Breeding	225	0	0.000%	0	0%

Legend. UFT: Unfit to Continue Travel.

There was an association between species transported and year ($X^2 = 40.25$; $p < 0.001$), season ($X^2 = 38.28$; $p < 0.001$), month ($X^2 = 67.19$; $p < 0.001$), provenance ($X^2 = 751.88$; $p < 0.001$), nationality of transport company ($X^2 = 1200.74$; $p < 0.001$) and destination ($X^2 = 62.67$; $p < 0.001$) (Figure 3, Figure S2).

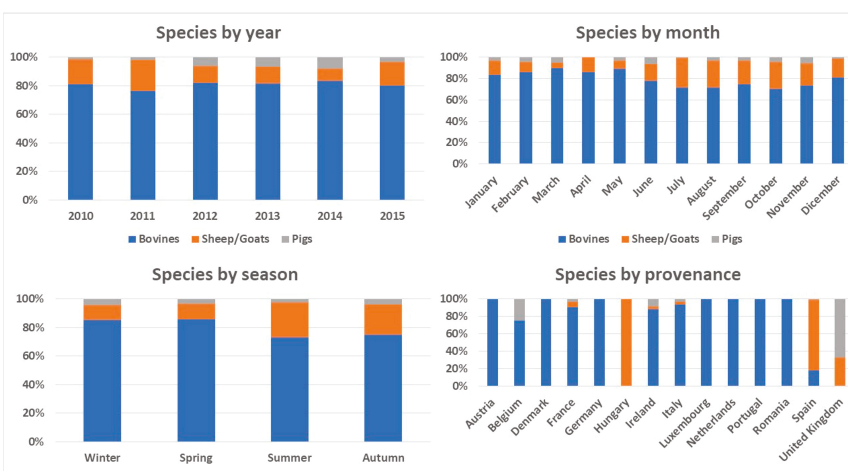

Figure 3. Association between species and year, month, season, provenance, nationality of transport company and destination.

Consequently, there was also an association between category transported and year (X^2 = 841.29; $p < 0.001$), season (X^2 = 90.83; $p < 0.001$), month (X^2 = 191.79; $p < 0.001$), provenance (X^2 = 1603.70; $p < 0.001$), nationality of transport company (X^2 = 2114.33; $p < 0.001$) and destination (X^2 = 192.41; $p < 0.001$). There was a higher number of trucks transporting sheep/goats in June, July and August, i.e., in summer, than in other months and seasons of the year. Sheep/goats came mainly from Spain and Hungary.

In the univariate logistic model, species of animal being transported and space allowance were the only predictive variable which proved to be associated with a welfare problem (X^2 = 5.780; df = 2; $p = 0.049$, and X^2 = 8.982; df = 1; $p = 0.003$, respectively) (Table S1). Trucks transporting sheep/goats were four times more likely to be associated with a case of DOA, DCP, or UFT than those transporting bovines. For a unit increase in space allowance, the odds in favor of the welfare problem occurring decreased by a factor of 0.24 (OR: 0.24; CI: 0.10–0.61) (Table 4). In the multivariate model, only space allowance remained significant ($p < 0.05$).

Table 4. Results of the univariate regression analysis between welfare problem (trucks reported with a case of DOA, DCP or UFT) and species of the animal being transported. Data were collected from trucks (n = 1391) transiting across a control post in Southern Italy from 2010 to 2015.

Variable	Category	Estimate	SE	OR	95% CI	*p*
Space Allowance		−1.400	0.469	0.24	0.10–0.61	0.003
Species	Bovines	Ref		Ref		
	Sheep/goats	1.437	0.610	4.20	1.27–13.91	0.018
	Pigs	−4.3	10.3	0.01	0–69,665	0.672

Legend. SE: standar error; OR: odds ratio; CI: confidence interval.

At the GLM, the effect of the year was not significant ($p = 0.072$) on NATT. While the number of transported animals per truck varied significantly depending on species (pigs 106.7 ± 4.7, sheep/goats 268.7 ± 2.2, cattle 41.0 ± 1.0; $p < 0.001$), the effect of the other predictive variables associated with species resulted in significant differences on NATT (Table S2).

4. Discussion

This study documented transport-related mortality and prevalence of animals considered unfit for transport for farm animals (i.e., cattle, sheep/goats, pigs) on a stopover at a control post in Southern Italy using reports of OVs. This study reports on the number of animals which died and were judged unfit to continue travel by OVs in compliance with Section 5 of Annex 1 of Council Regulation (EC) No. 1/2005, thus providing the first report containing statistics on farm animal movements from Northern Europe to the Balkan Peninsula. Considering that only 0.8% of the shipments was associated with one of the considered welfare issue (i.e., DOA, UFT), and that the Eurobarometer [21] reports that the perception of animal welfare has increased among European consumers, our data may help consumers gain knowledge of live animal transport within Member States.

The animals on the stopover at our CP were mainly travelling to Greece, a country which relies heavily on meat and dairy imports [22]. Animals were being transported for different purposes: for the final fattening period before slaughtering in the destination countries, for breeding, for direct sale or for slaughter. The number of vehicles stopping at the CP dropped significantly after 2012, probably due to the economic crisis in Greece [22]. The reasons for the great demand for live animal transport have been analyzed by the European Union [23] and include a high level of demand for fresh meat; use of indigenous slaughterhouse facilities; use of by-products (such as skin and offal) from the slaughter process; poor meat transport, refrigeration and storage capacity; use of religious slaughter rituals in some EU-countries. The EUR-Lex (1998) states that even though animal welfare suggests slaughtering an animal close to its origin and then transporting the carcass to its final destination, individual Member States have such conflicting interests that no consensus can be reached on the issue. Safeguarding animal welfare during transport is therefore crucial.

In our study, the majority of the trucks came from France, transporting mainly bovines. This was expected, since France exports millions of stocker calves to other EU countries each year for fattening [7]. Spain was the second most common provenance, but these vehicles were transporting sheep/goats; this was also expected, since Spain has the second-highest number of sheep (a total of 18,136,050) amongst European Union Member States [24]. The transport companies were mainly Greek, probably because the final destination was Greece. Indeed, Greece has the highest per capita consumption of sheep meat in Europe and is a major importer of sheep [25]. However, such data lead us to reflect on the importance of live animal transport for the Greek economy and of the training of Greek livestock hauliers. Europe is currently the only part of the world where hauliers are required to undergo training, but according to a 2007 survey, only 33.3% of the hauliers had attended training courses provided either by the Local Health Authority or trade organizations [26]. More recently, a survey proved the need of additional education and training for livestock drivers in Denmark [27]. Consequently, courses for transporters should be implemented and promoted worldwide, since training for all those involved in live animal transport has been identified as a key factor for improving welfare outcomes among transported animals [28].

Mortality is often used as an animal welfare indicator, since it is beyond doubt that such deaths are preceded by a period of suffering and poor welfare [14]. In relation to transport, mortality has often been reported as DOA, down on trailer or before weighing and total dead [29]. In our case, DOA, DCP and total dead showed an overall mortality rate within the range reported in the literature [25]. In our study, the mortality rate for bovines was similar to the rates reported in Canada (0.011%) [16] and in Czechia (0.012%) [30]. Contrastingly, the mortality for sheep/goats was outside the range (0.006–0.018%) reported in literature [31]. The highest mortality rate was found in lambs, probably because they are more likely to suffer from protracted transport stress than adult sheep and because they are more likely to be transported in large loads [31].

The mortality in pigs was 0%, lower than reported by Averós et al. in 2010 [17]. However, the latter authors focused on weaned piglets, while in our study fattened or breeding pigs were being transported, so they were older and often being transported in single compartments. All these factors may have helped the animals cope better with the long journey, thus reducing the overall mortality rate [14]. However, the number of pig transport records in this study is low, so this should also be taken into account when interpreting our data.

The morbidity rate was lower than reported in literature [2,16,29] and the reasons for this discrepancy may be due to a variety of factors. Firstly, the criteria for assessing morbidity in the above mentioned studies varied from one author to the next, while our reports were filed by OVs based on the criteria set out in Council Regulation (EC) No. 1/2005 for the fitness for transport. The OVs stopped only those animals showing severe injuries and diseases; consequently, no minor injuries or pathologies were counted in our dataset, thus reducing the morbidity rate. Secondly, the enforcement of a correct assessment of fitness for transport pre-departure may have played a role in reducing morbidity. While Marlin et al. (2011) reported that many transport related injuries assessed at unloading were due to animals' pre-existing poor health and welfare status, the number of animals stopped at departure was not considered in our dataset. Finally, the majority of publications assess morbidity at the slaughterhouse, while our data are the first to record it at a CP; our animals could have had time to recover from the long journey at the CP, being milked and humanely handled by the technicians working there. It has recently been shown that sheep rested for at least 16 h at a CP recovered from the stress, dehydration and fatigue induced by a 29-h journey [11]. The goal of CPs is indeed to give the animals the time to recover from the effects of a long journey before being loaded and transported again. Unfortunately, there are very few scientific studies on the effects of different management systems and resting period at CPs [10,11]. Our data could not therefore compared with the literature. Further studies should be conducted comparing transport related morbidity at different CPs and in more countries.

In this study, sheep/goats were found to be more likely affected by transport than the other types of animals. It has been reported that sheep usually cope better with transport than other species [31], though there have been occasional reports of high mortality among single loads of animals during journeys that are non-compliant with regulations, such as an infamous case of 65 deaths out of 400 sheep being transported from Poland to France [31]. Similarly, our rate may have been affected by the fact that 14 out of 214 lambs were found dead on arrival in a single vehicle, which proved to be not compliant with EU regulation on space allowance and fined for overcrowding. It has been assumed that transport is less tiring for sheep/goats because as opposed to cattle and horses, both lambs and adult sheep lie down during transport [14]. However, studies have documented that transport reduced resting and rumination behaviours of lambs which showed signs of stress soon after loading and high levels of dehydration and weight loss after journeys lasting 12 h [31,32]. The number of inspections should be increased on trucks transporting lambs in Europe.

Overcrowding has been identified as a risk factor for transport-related health and welfare issues and it was the most frequent infringement observed during vehicle inspections carried out between 2001 and 2010 at the border between France and Italy [7]. Council Regulation (EC) No. 1/2005 gives a stocking density range for bovines and sheep/goats expressed in m^2/animal, which vary according to the category of animal transported. The range for lambs goes from 0.20 to 0.30 m^2/animal. However, it has been shown that stocking density should not be expressed in square meters per animal but in square meters per 100 kg to allow lambs to lie down and cope better with transport stress, and that lamb health and welfare may be affected by high stocking density [32]. Our data may be useful to implement the existing European Transport Guidelines and further studies should be performed to determine the optimal stocking density of lambs transported over long journeys within Europe.

Journey duration was confirmed as the most important risk factor in the development of transport-related diseases [33]. Surprisingly, in our data set there was no association between welfare outcomes and provenance. One reason for this finding may be related to the fact that the journeys had to follow a fixed route which was checked and approved by OVs before departure. However, it may also be due to the fact that we could not ascertain the exact journey duration in our dataset and that we used provenance as a predictive variable. Further studies should be conducted on a larger dataset using journey duration as a predictive variable to ascertain our findings.

Season and month have been identified as risk factors for transport-related mortality [29,30,33]. However, in our study, neither season nor month were significant. Our results are similar to those reported for pig transport by Gosálvez et al. 2006 [34], who explained their results by Spanish pig hauliers taking precautions to protect animals from extreme conditions, such as undertaking journeys at night, reducing loading densities and showering animals. Apart from those precautions taken by the hauliers, our results might also be due to the development of new vehicles. Those vehicle must have appropriated ventilation systems, temperature monitoring and recording systems [35], to comply with the new standards required by Council Regulation (EC) No. 1/2005 for journeys exceeding 8 h. These new technologies may have enhanced the welfare of the transported animals, assuring thermal comfort throughout the year. Overall, our data should be interpreted with caution and confirmed using a larger dataset.

The NATT was affected by many of the factors studied, in particular by species and category. There was a high average number of transported animals among vehicles transporting sheep/goats or younger—and consequently less heavy—animals. Since species and category were associated with other factors such as provenance, nationality of the transport company and destination, the effects of these latter factors on the number of transported animals per truck should be interpreted as a consequence. For instance, since sheep/goats came mainly from Spain and Hungary, all trucks coming from those countries contained higher than average numbers of transported animals. The association between species and category with season or month could, however, reflect consumer trends (e.g., eating more meat in winter; traditional consumption of lamb during religious events) and the typical commercial life of each animal. For instance, lambs are usually born in spring, weaned after three months and are then shipped to feed lot or slaughter; this could explain why we found that the transport of sheep/goats was positively associated with summer months.

Our data should be considered preliminary because this study was limited by a number of factors. Firstly, due to the small number of events (welfare problem occurred) the logistic model was likely to suffer from small-sample bias. Secondly, the surveillance reports were cumulative by truck, which made it impossible to perform a proper risk analysis based on single transported animals. Thirdly, as the assessment of welfare outcomes was related only to death and severe pathology, in compliance with Section 5 of Annex 1 to Council Regulation (EC) No. 1/2005, many minor injuries and pathologies were not recorded and consequently were not analyzed in this manuscript. Finally, as previously discussed, our dataset was unable to include the health condition of the animals before the journey, the exact duration of the journey and stocking density expressed in m^2/100 kg. Notwithstanding these limitations, this is the first paper reporting statistics based on surveillance reports filed by official veterinarians for livestock transported from Northern Europe to the Balkan Peninsula and transiting through a control post in Southern Italy.

5. Conclusions

This was the first study documenting farm animal movements from Northern Europe to Greece transiting through a control post and their subsequent welfare outcomes as assessed by OVs. Only 0.8% of the trucks reported a case of mortality or morbidity and lambs transported with minimal space allowance proved to be at higher risk of poor welfare. Due to the aforementioned limitations, our findings should be considered preliminary. Further studies should be carried out in more CPs and in other countries to confirm our data. Further studies should also assess welfare on individual transported animals to identify risk factors and to analyze welfare outcomes among transported animals, comparing the welfare assessment based on official reports versus the scientific welfare assessments suggested in more recent literature.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-2615/8/9/155/s1, Figure S1: Trends of the number of trucks by year and species and by month and species, Figure S2: Association between species and nationality of Transport Company and destination, Table S1: Wald test *p*-values generated from univariate regression analysis, Table S2: Results of the GLM with year, month, season, species and category as factors and number of animals transported per truck (NATT) as dependent variables. Results expressed as Least Square means ± standard error.

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animals

MDPI

Communication

An Evaluation of Two Different Broiler Catching Methods

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Simple Summary: Catching is the process that transfers birds from the poultry house to the transport modules. The catching process and its associated handling may lead to stress, injuries, mortality and reduced welfare for the animals. The aim of this pilot study was to investigate the effect of two manual broiler catching methods. Broilers were either caught by both legs and carried inverted to the drawers or caught under the abdomen and carried in an upright position. Effects of catching method on crating time, number of animals in the drawers, wing and leg fractures, animals on their back in the drawers and broilers dead-on-arrival were investigated. The results showed that the abdominal and upright method was faster and gave a lower and more consistent number of birds per drawer. In addition, this method tended towards fewer wing fractures. No broken legs, birds on their back in the drawers or broilers dead-on-arrival were observed in the study. Catching is a critical phase in the pre-slaughter chain, and this study shows that the catching and carrying method affects broiler welfare.

Abstract: Catching is the first step in the pre-slaughter chain for broiler chickens. The process may be detrimental for animal welfare due to the associated handling. The aim of this pilot study was to compare two different methods to manually catch broilers: Catching the broilers by two legs and carrying them inverted (LEGS) or catching the broilers under the abdomen and carrying them in an upright position (UPRIGHT). Wing and leg fractures upon arrival at the abattoir, animal density in the drawers, birds on their back, broilers dead-on-arrival and time to fill the transport modules were investigated. The results showed that mean crating time was shorter in the UPRIGHT method ($p = 0.007$). There was a tendency for more wing fractures in broilers caught by the LEGS ($p = 0.06$). The animal density in the drawers was lower and with a smaller range in the UPRIGHT method ($p = 0.022$). The results indicate that catching the broilers under the abdomen in an upright position may improve broiler welfare in terms of fewer wing fractures, more consistent stocking density in drawers and potentially reduced loading time.

Keywords: animal welfare; broiler; catching; pre-slaughter chain; poultry; wing fractures

1. Introduction

Catching is the first step in the pre-slaughter chain of broiler chickens [1]. In this process, the broilers are transferred from the floor of the broiler house into transport modules, which are then loaded onto vehicles for transport to the abattoir.

The catching process may be performed mechanically or manually [2]. Under commercial manual catching conditions, the broilers are commonly caught and carried by one leg, with three to five broilers in one hand and one or two in the other hand [3,4]. The broilers are carried inverted to the transport module, which is usually a container with several drawers where the animals are crated. The catching and crating process may cause stress [1,2,5,6], injuries, and mortality [7–9] and hence, compromise the welfare of the broiler chickens. The Humane Slaughter Association and EU's "Animal Transport Guides" (Consortium of the Animal Transport Guides Project) recommend catching birds individually with a grip over the wings, in an upright position [3,10]. The same catching method is recommended in Brazil due to a reduced risk of injuries for the birds [11]. Catching and carrying in an upright position can reduce handling stress, measured as corticosteroid concentration [12,13]. Furthermore, handling in an inverted position leads to prolonged tonic immobility, which is a commonly used measure of fear [14]. If the birds are to be caught and carried inverted, several organizations recommend that the best practice is to catch the birds by both legs [3,15]. Approximately four billion broiler chickens are kept for meat production in the European Union [16]. The number of birds implies that injuries and poor welfare even in a small portion of the poultry population affects a large number of animals [17]. Hence, reducing stress and injuries during catching and carrying is of utmost importance.

Knowledge, skills and training of personnel involved in handling animals are fundamental in improving the welfare of commercial livestock [18]. However, there is little scientific literature concerning different manual catching methods of poultry. It is, therefore, necessary to improve scientific knowledge regarding how different manual catching methods affect broiler welfare. This knowledge can be used by the industry to ensure that catchers are trained according to best practice. The aim of this study was, therefore, to assess the effects of two different manual catching methods; catching by two legs or catching under the abdomen in an upright position. Since catching may affect animal welfare, as well as put a strain on the workers, logistics and economy, the following indicators were recorded: Crating time of individual transport modules, the number of birds in each drawer, wing and leg fractures, birds on their back in the drawers observed in lairage, and broilers dead-on-arrival (DOA).

2. Materials and Methods

The pilot study was designed to evaluate two different methods of manual broiler catching. LEGS: The broilers were caught by two legs and carried upside-down (Figure 1). Usually, this method allowed two or three broilers in one hand and one in the other hand (range: Two to four birds in one hand, one to two in the other).

Figure 1. Catching and carrying broilers by a grip in both legs.

UPRIGHT: The broilers were caught under the abdomen (Figure 2) and carried in an upright position to the transport modules. Mostly two broilers were caught and carried in each catch by the upright method (range: One to two birds per catch; a catch refers to each time the catchers pick up birds and place them in the drawer).

Figure 2. Catching under the abdomen and carrying the broilers upright.

The study was carried out under commercial conditions in one major broiler producing region in Norway (Trøndelag). Two flocks from two different farms were enrolled. The two flocks were of different hybrids; one flock was a conventional fast-growing type (Ross 308), flock size 30,000 broilers, slaughtered at the age of 33 days and 1328 g (mean carcass weight for the flock, estimated live weight 2000 g) and the other flock was a slower growing hybrid (Hubbard JA 787), flock size 16,800 broilers, slaughtered at the age of 44 days and 1539 g (mean carcass weight for the flock, estimated live weight 2300 g). Both flocks were mixed sex and fed ad-libitum. Both broiler houses were from the same manufacturer, had the same size and were equipped with the same ventilation system.

The study sample consisted of 3951 broilers from both houses in total; 2010 caught by LEGS (1031 broilers from the conventional hybrid and 996 broilers from the slower growing hybrid) and 1941 by UPRIGHT (969 broilers from the conventional hybrid and 955 broilers from the slower growing hybrid). This implies that the majority of the broilers in the two flocks were not included in the study. Both catching methods were evaluated in both flocks.

The sampling was carried out in dimmed lighting at the start of the catching process. Only the first eight transport modules in each house were included in the study; the first four modules with LEGS, then the next four modules with UPRIGHT. The birds were caught randomly for each method. The two broiler flocks were caught two subsequent nights by the same catching team, consisting of the same four professional catchers. The team consisted of two women and two men; all had catching as their primary occupation. They had no standard catching method, sometimes by one leg, sometimes by two legs and sometimes upright by abdomen. For this study, they were trained to catch both by LEGS and by UPRIGHT methods. The training consisted of theoretical instructions along with practical demonstrations. For LEGS, the only instruction was to catch by both legs for each bird. For UPRIGHT, the only instruction was to catch under the abdomen and carry the broilers in an upright position. The instruction did not regulate the maximum number of birds allowed per catch. All catchers performed both methods in this study.

Both flocks were caught late night/early morning. The size of the transport modules was identical for both hybrids and methods (Stork®, 2.43 × 1.30 m [length × width], eight open-topped drawers in a module, a planned minimum of 200 cm^2 per kg live bird). The transport modules were marked according to catching the method, and the eight modules were loaded on the same vehicle. The time to fill each transport module with birds was recorded. The modules were loaded in and out of the broiler house with a forklift. The truck driver placed all modules as close as possible to the broilers as per normal industry practice. This process was consistent for both methods and both flocks.

The journey time from farm to abattoir was 30 min for the conventional flock and 10 min for the slower growing flock. Immediately after the transport modules arrived at the abattoir, the broilers were manually lifted out of the modules and investigated in lairage for wing and leg fractures, birds on their back in the drawers and DOA. The birds were assessed by two of the researchers at the same time. Injured birds were immediately stunned with blunt trauma to the head, followed by cervical dislocation. The wing fracture criteria were: open or closed fractures, dislocated wings and detachment of the epiphyseal plates with visible bleeding around the elbow joints [9]. In addition, the number of animals per drawer was recorded. These investigations were performed in lairage and not on the farm, due to better lighting and protection against low temperatures at the abattoir. The assessors of the birds in lairage were aware of the catching method.

Data were entered into an Excel spreadsheet and transferred to STATA 14.2 (College Station, TX, USA). All observations were inspected for deviations and missing data before the analyses. The distribution of the continuous variables (crating time and stocking density) were inspected visually by histograms and by summary statistics. Both variables were approximately normally distributed. Simple linear regression was used to compare means of catching and crating time, the number of birds per cage, DOA and fractures by hybrid and catching method. An interaction term was included in the regression analyses to evaluate whether the univariable relationships between stocking density and hybrid, as well as catching time and hybrid were dependent on the simultaneous effects of hybrid and catching method. Residuals were predicted and visualized by histograms. Since wing fractures were either present or absent in each module, this variable was treated as categorical. Thus, for wing fractures, simple logistic regression was used to study the effect of birds per cage, hybrid, crating time, and catching method. Hybrid was included as a covariate in the study of effects of birds per cage and crating time on wing fractures. Residuals (linear regression) were predicted and displayed on normal quantile plots.

3. Results

For a summary of the main results, see Table 1. For a comparison of the two methods, broken down to flock, see Table 2.

Table 1. Outcome measures of upright and leg catching methods, trialled in one conventional and one slow-growing flock of broilers.

Indicators	Mean	Conventional Hybrid [1], N = 2000	Slower Growing Hybrid [2], N = 1951	*p*-Value, Conventional vs. Slow-Growing Hybrid	LEGS, N = 2010	UPRIGHT, N = 1941	*p*-Value, LEGS vs. UPRIGHT
Crating time [3]	241	219	264	0.001	252	231	0.007
Number of birds per drawer	30.87	31.67	30.06	0.001	31.25	30.48	0.022
Wing fractures [4]		2	6	0.164	7	1	0.060
Leg fractures	0	0	0	-	0	0	-
DOA	0	0	0	-	0	0	-

[1] Age 33 days, mean estimated live weight 2000 g; [2] Age 44 days, mean estimated live weight 2300 g; [3] In seconds per transport module; [4] Total number of birds with fractures.

Table 2. Comparison of catching methods, broken down to flock.

	LEGS			UPRIGHT		
Indicators	**Conventional Hybrid [1]**	**Slower Growing Hybrid [2]**	***p*-Value**	**Conventional Hybrid [1]**	**Slower Growing Hybrid [2]**	***p*-Value**
Mean crating time [3]	242.8	261.1	0.053	202.3	260.0	<0.0001
Mean number of birds per drawer	32.2	30.3	0.0001	31.1	29.9	0.0124
Wing fractures [4]	2	5	<0.001	0	1	<0.001

[1] Age 33 days, mean estimated live weight 2000 g; [2] Age 44 days, mean estimated live weight 2300 g; [3] In seconds per transport module; [4] Total number of birds with fractures.

3.1. Crating Time

Overall, the mean time to fill the transport module was 241 s (range 161–340; SEM 3.84). There was a significant difference in time to fill the transport modules between the two hybrids. For the conventional fast-growing hybrid, the mean time to fill was 219 s (range 161–270; SD 34.03) and 264 s (range 219–340; SD 40.12) for the slower growing hybrid (p = 0.001). The difference in crating time between the two hybrids when the interaction between hybrid and catching method was included was significant (p = 0.048). Mean crating time was significantly shorter for the UPRIGHT method; the mean time to fill a transport module by LEGS was 252 s (range 188–340; SD 41.48), versus 231 s (range 161–313; SD 43.28) for UPRIGHT (p = 0.007).

3.2. Number of Birds Per Drawer

The mean number of birds in the drawers was 30.87 (range 25–36; SD 1.90). For the conventional fast-growing hybrid, the average was 31.67 birds (range 27–36; SD 1.80) (space allowance: 220 cm^2/kg estimated live weight), and for the slower-growing hybrid, it was 30.06 birds (range 25–35; SD 1.64) (space allowance: 197 cm^2/kg estimated live weight). The difference in the number of hybrid animals per drawer was significant (p = 0.001). The interaction between hybrid and catching method was not significant (p = 0.273). The average number of birds in each drawer was 31.25 for LEGS (range 25–36; SD 2.10) (space allowance, regardless of hybrid: 204 cm^2/kg, estimated live weight), and 30.48 birds for UPRIGHT (range 26–33; SD 1.59) (space allowance, regardless of hybrid: 211 cm^2/kg, estimated live weight) (p = 0.022).

3.3. Wing Fractures

Wing fractures were not a common finding in this study; in total eight wing fractures were observed upon investigation in lairage. Two fractures were observed in the conventional fast-growing hybrid and six fractures in the slower growing hybrid (p = 0.164). Seven wing fractures were observed in broilers caught by LEGS, one fracture for broilers caught by UPRIGHT. There was a tendency for higher odds (OR = 7.74) of wing fracture for LEGS versus UPRIGHT (p = 0.06).

3.4. Other Welfare Indicators

Upon examination in lairage, none of the broilers included in this pilot study were dead-on-arrival or on their back in the drawers. Likewise, no leg fractures were observed.

4. Discussion

Despite limitations in the number of animals enrolled in the study, the catching method was observed to affect several of the parameters recorded. Catching the broilers under the abdomen in an upright position was a faster method, gave slightly fewer wing fractures and gave a more consistent animal density in the drawers, compared to catching broilers by two legs and carrying them inverted to the drawers. A comparison with one-leg catching was not performed since this method is prohibited in Norway [19].

There was a tendency for fewer wing fractures observed in broilers caught upright under the abdomen. An overall low prevalence of wing fractures was recorded (0.20%). Since the wings were examined for fractures in the lairage, a distinction between fractures due to catching and due to transportation could not be made. However, Jacobs et al. [7] investigated wing fractures post catching and post lairage. They found 1.88% wing fractures after catching and 1.90% in lairage, indicating that the fractures are typically attributable to catching and not to transportation. Available literature on wing fractures registered prior to processing is sparse. Kittelsen et al. investigated wing fractures prior to stunning and found 0.8% wing fractures in the observed broilers [9], a slightly higher number than observed in the current study. Langkabel et al. found no significant differences in the number of wing fractures between one-leg and two-leg catching [4], however, it was reported that the animals appeared to be more restless and performed more wing flapping during two-leg catching. In the current study, no one-leg catching was investigated, but catching by two legs gave more wing flapping than catching upright under the abdomen (anecdotal observation). The results from both studies indicate that the activity of the broilers during carrying did not affect the prevalence of wing fractures. It can be hypothesized that the actual crating process represents a larger risk for wing fractures than the carrying. This may explain the tendency for fewer fractures in broilers caught upright under the abdomen, since this method had more controlled placements of the birds in the drawers. There is a possibility that the catcher's behavior and handling of the birds were subject to observer effect. However, the catchers did not know which indicators would be assessed. The observation may have affected all handling equally, but it does not explain the differences between the catching methods.

Crating time was included in the study since the total time taken to catch an entire flock is important for broiler welfare, as well as the logistics and economy of the industry. Prolonged catching will influence time without feed and water, and therefore cause stress to the birds [20,21]. Crating time was directly and simultaneously influenced by both the hybrid and the catching method. Catching of the commercial fast-growing hybrid was quicker compared to the slower growing hybrid, regardless of method. This may be due to the weight of the slow-growing hybrid. The results also show that catching the broilers UPRIGHT, either one or two birds at a time, was faster than getting a grip of both legs of the animals, even though more birds could be collected in one catch with the two-leg method. In accordance with Langkabel et al. [4], the catchers were observed to fumble more to collect both legs than when they lifted one or two birds upright under the abdomen (anecdotal observation). This study did not consider how the level of practice and experience that the catchers had prior to the study may have influenced the crating time. The catchers perceived the abdominal catching to be most exhausting, since this method allowed fewer birds per catch and therefore more squats. The method's physical load on the catchers may affect crating time negatively if this method was performed in the entire flock, since prolonged depopulation time may increase stress and time without feed and water. However, it must be noted that too rapid handling also may be detrimental to bird welfare, as this may increase the risk of injuries, and as such, rapid handling by itself is not a goal. Few broilers were included in this study, compared with a commercial catching of larger flocks. Therefore, further studies are needed to assess the effect of the catching method on the crating time.

The number of broilers per drawer was higher when the birds were caught by LEGS, compared to UPRIGHT. In addition, the range in the number of birds per drawer was higher, making the crating density inconsistent with LEGS. The animal density is an important parameter during transport: If the density is too high, it gets more difficult for the birds to tackle high or low on-board temperatures and dead-on-arrivals may be the outcome [22–24]. The reason for this is that the mechanisms broilers use for cooling are less effective at higher densities [25]. Therefore, it is important to obtain the optimal density in each drawer. The reason for the significant difference in animal density between the two catching methods may be uneven "catches" and a larger range in the number of animals per catch. Especially with LEGS, the number of birds per catch varied between catches and between the catching personell. This made it difficult to keep track of the number of birds per drawer, especially since two catchers filled one drawer at the same time. The problem with crating density will likely be solved with more experience or a catcher designated to count the broilers.

In this pilot study, the catching method was applied randomly. However, due to the limited size of the study, LEGS was performed prior to UPRIGHT in both flocks. This may have affected our results since the catchers could have been more tired when starting abdominal catching. However, only a small proportion of the flocks were a part of this study, and the depopulation started with these two methods. Approximately 1000 broilers were caught by LEGS, followed by 1000 broilers by UPRIGHT in each flock. This equals approximately 3.33% and 5.95% of the flocks, respectively, which is only a small proportion of the animals that the catchers are used to handling each night. The impact of the catching method used when observers left the broiler house could have been used as a control, but this was not possible since the recording of wing fractures in lairage were very time-consuming. Including additional registration on controls would be negative for animal welfare in this study, as it would prolong the total crating time, and the time without feed and water for the entire flocks. In this study, the flocks were categorized as different hybrids. This may be misleading as there were several other factors that may have affected the results, e.g., live weight, management. This should be further investigated in future studies.

In previous studies on catching, bruising is included as a welfare indicator. In the current study, all registrations were observed in lairage, prior to the slaughter process. Bruising can only be observed after defeathering and was therefore not included as an indicator in this study. Several questions need to be addressed in a follow-up study with a larger sample size; these include the effect of crating time if the methods are applied in large flocks and more welfare indicators (e.g., stress evaluation and bruising).

5. Conclusions

Catching is a critical phase in the pre-slaughter chain, and this study shows that the catching and carrying method affects broiler welfare. One major finding is that there is a strong tendency towards more wing fractures when the birds are caught by two legs. In addition, the time to fill a transport module was less with the UPRIGHT method and resulted in a lower and more consistent animal density in the drawers compared to catching by two legs. Clearly, due to the small sample size, further studies are needed to form the basis for improved and more animal-friendly catching routines. It must be noted that both methods are likely to slow down the catch rate compared to one leg capture and this may have economic implications for the industry due to increased personnel costs. The consequence of this is unknown. However, catching by one leg is prohibited and alternative catching methods need to be implemented.

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animals

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Qualitative Behavioural Assessment as a Method to Identify Potential Stressors during Commercial Sheep Transport

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Simple Summary: Land transport is a common and unavoidable experience for most livestock, yet it remains a health and welfare concern. From the animals' perspective, transport involves mixing with other animals, novel experiences, and prolonged standing, often after periods of water and feed withdrawal ('curfews'). Although the physical effects of transport have been studied, often by the impact on meat quality, the effects on the mental well-being of sheep are unknown. The aim of this study was to identify factors that influence the behavioural expression of sheep undergoing land transport, using observers who were blinded to the experimental treatments to score the animal's body language during land transport. Various vehicle crate types, deck positions, sheep breeds and point of origin were compared. All treatments were variations on current commercial transport, and therefore stocking density was similar between the vehicles as per regulatory requirements, but truck designs varied. This study supports using the scoring of behavioural expression to assess sheep welfare during transport.

Abstract: Land transport is an unavoidable experience for most livestock, yet there is limited research comparing animal welfare under different conditions. We video recorded sheep responses during short (2 h) commercial road transport journeys. Using Qualitative Behavioural Assessment, observers (blinded to the treatments) scored the behavioural expression of sheep and reached significant consensus in their scoring patterns ($p < 0.001$). There were also significant effects of vehicle crate design (sheep transported in a 'standard' crate were more *calm/relaxed* than those transported in a 'convertible' crate), deck position (sheep on upper decks were more *curious/alert* than those on lower decks), and sheep breed (fat-tail sheep were more *agitated/distressed* than merino sheep) on observer scores. We only found marginal differences for sheep originating from feedlot or saleyard. Significant effects of vehicle driver (included as a random factor in all but one of our analyses) suggest driving patterns contributed to demeanour of the sheep. Finally, the fourteen drivers who participated in the study were asked their opinions on livestock transport; none of the factors we tested were identified by drivers as important for sheep welfare during transport. This study supports the use of qualitative measures in transport and revealed differences that could inform truck design.

Keywords: qualitative behavioural assessment; QBA; sheep; transport; behaviour

1. Introduction

There is strong public interest and much research aimed at ensuring the welfare of transported livestock is optimal. The wellbeing of sheep during road transport can be influenced by several factors, including loading and unloading [1], stocking density [2,3], temperature and humidity [4,5], driving behaviour [6], vibration and noise, change in acceleration and cornering [4,6] road type [7] prolonged standing, unfamiliar mixing, novel environment, hunger, thirst and fatigue [4]. Additional factors, such as design of the vehicle and/or breed or past experience of the animals, may be less obvious in terms of their impacts on animal wellbeing but are nevertheless important to consider when striving for industry best practice.

Much research has examined the physiological responses of sheep to varying transport conditions. For example, increased cortisol and heart rate have been recorded for longer transport trips with extended curfew periods [8] and frequent changes in acceleration [9]. This research indicates the stress associated with some major transport risk factors, but is invasive, hence there is still a demand to develop practical assessment measures that can be applied in the field. Additionally, there are few measurable parameters that reflect the affective state, or how an animal is coping over time that do not add further distress to the individual upon collection.

Qualitative Behavioural Assessment (QBA) is an on-farm assessment tool [10–12] and has been shown to be a meaningful indicator for on-farm welfare in sheep [13]. QBA examines the expressive body language of animals as they interact with their environment; animals are scored against qualitative terms (e.g., *anxious*, *fearful*, *calm*) that describe these interactions. Qualitative behaviour explores not *what* the animal is doing, but *how* the animal is performing a specific behaviour, and it can be argued that stockpersons routinely use such expressive terms to manage their animals' welfare state. QBA can therefore provide animal welfare-relevant measures that are non-invasive, relatively quick, and inexpensive, that can be applied in production scenarios where intensive animal measures are difficult to implement. The method has been validated under a range of experimental conditions (reviewed by [14]) and QBA scores correlate well with physiological stress responses of both sheep [15,16] and cattle [17,18] under experimental conditions during road transport. Furthermore, observers, blinded to treatments are able to distinguish between treatment groups based on the animals' behavioural expression [14], demonstrating that QBA is a repeatable, objective and valid measure of animal behavioural responses.

Two important factors that influences welfare of transported animals are the characteristics of the vehicle itself and the way the vehicle is driven [19]. Vehicle design varies in response to environmental conditions and may impact ventilation system, suspension and flooring. In Australia, livestock are frequently transported for long distances by multi-decked open-sided trucks, relying on free ventilation due to vehicle movement. Poor ventilation may lead to heat stress, particularly in hot climates, as temperature inside the vehicle may rise within the standing vehicles (i.e., during loading) [20]. Poor suspension can affect animal welfare, as excessive vibration has been shown to lead to fear and muscle fatigue in calves [21]. Non-slippery flooring is essential to prevent animals falling, and vehicle movement and driving quality may cause distress to sheep as animals are forced to continually balance the effect of movement forces [22].

The aim of this study was to examine the behavioural responses of sheep being transported in two vehicle types and to identify potential risk factors during commercial road transport. Using Qualitative Behavioural Assessment (QBA), we compared the behavioural expression of sheep transported using two types of vehicles routinely used in Australia: 1. a 'standard' truck design that has either a three-deck or a four-deck trailer and 2. a newer 'convertible' truck design with a trailer holding four decks for sheep (but which can convert to two decks for cattle) (Figure 1). Both designs have a maximum length of 27.5 m and contain some differences in the compartment or 'crate' in which the livestock travel. The convertible crates had slightly narrower ventilation slots and straight sides to the upper deck, whereas the standard crate had a curved side rail of the upper deck (as seen in Figures 2 and 3).

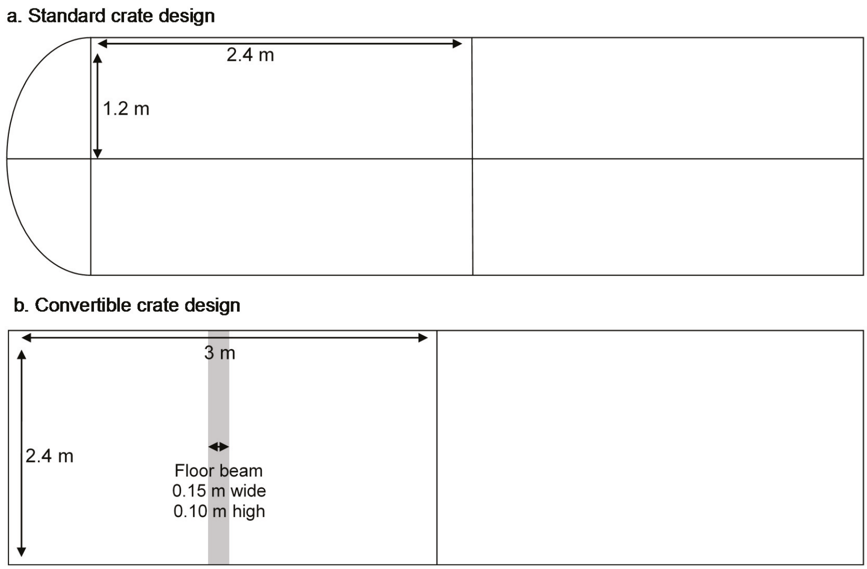

Figure 1. Schematic diagram of vehicle designs (**a**) standard and (**b**) convertible crates.

(**a**)

Figure 2. *Cont.*

(**b**) (**c**)

Figure 2. View showing (**a**) sides of a standard 4-tier commercial sheep transport vehicle, (**b**) sides of the top deck of a convertible, and (**c**) top deck of a standard crate.

(**a**) (**b**)

Figure 3. Views of merino sheep on the top deck in a (**a**) standard crate and (**b**) convertible crate. Note the curved side rail in (**a**) and straight side rail in (**b**).

The four factors examined in this study were: the design of trailer crate, the deck level of the crate, the breed of sheep, and their point of origin (reflecting immediate pre-transport handling). Differences in QBA scores for sheep between these vehicle types may be useful as an indicator of potential focal areas that require further investigation to improve livestock well-being during commercial transport.

2. Materials and Methods

2.1. Animals and Transportation

Sheep were video recorded (Figure 2) as a group during 52 short-haul (1–2 h) commercial road journeys. We filmed transport events opportunistically, and then later selected footage approximately 10 min after departure point that would allow us the opportunity to test for differences between experimental treatments (Table 1). Data was collected during routine sheep haulage over two months in Spring 2011 (Study A: Comparison of trailer crate design, breed of sheep and point of origin) and two months in Autumn 2013 (Study B: Comparison of the deck level within a crate).

The sheep filmed on all transport events were located in the fore or middle pen of the upper deck of the front crate, or the fore pen of the lower deck of commercial livestock transport vehicles registered in Western Australia. These pens were selected for their suitability for camera attachment and recording because it was unknown if adequate footage could be obtained from cameras mounted on these vehicles given the stocking densities and prevailing weather. Stocking densities and handling procedures for all transport events were at standard industry recommendations.

We carried out two studies, Study A compared the following: 1. trailer crate design, 2. sheep breed and 3. point of origin. Study B was an investigation of the effects of deck level for two trailer crate designs.

Table 1. Description of the treatment comparisons carried out. Bold text indicates the comparison made for each study.

Study	Vehicle Cate † S: Standard, C: Convertible	Deck U: Upper, L: Lower	Sheep M: Merino, F: Fat-Tail	Origin ‡	Number of Video Clips Viewed by Observers
A1 Vehicle crate	**S vs. C**	U	M	FL	*n* = 10, 10
A2 Sheep breed	S	U	**M vs. F**	FL	*n* = 5, 5
A3 Point of origin	S	U	M	**SY vs. FL**	*n* = 10, 10
B Deck level:					
B1 (SU-SL)	S	**U vs. L**	M	FL–W	*n* = 10, 10
B2 (CU-CL)	C	**U vs. L**	M	FL–W	*n* = 10, 10
B3 (SU-SL-CU-CL)	**S vs. C**	**U vs. L**	M	FL–W	*n* = 18, 18

†: Vehicles with the standard crate design had four 1.2 × 2.4 m W × L (2.9 m^2) pens per deck and a solid metal floor that was fixed in place. Vehicles with the convertible crate design had two 2.4 × 3.0 m W × L (7.2 m^2) pens per deck and a solid metal floor that was not permanently fixed in place. ‡: The routes taken included a period of continuous and stop-start driving, originating from either SY: saleyard (Muchea, Western Australia) to feedlot, or FL: from a registered premises feedlot (Baldivis, Western Australia) to a loading wharf (Fremantle, Western Australia).

2.2. Study A: Comparison of 1. Trailer Crate Design, 2. Sheep Breed and 3. Point of Origin

Study A1: we compared the behavioural expression of sheep transported on the upper decks of two vehicle types: a standard crate (S) and a convertible crate (C) (Table 1; Figure 3). In the convertible crate, two decks that carry sheep are designed to fold away up against the sides of the vehicle for the cartage of cattle; hence decks 1 and 3 have fixed floors and decks 2 and 4 (upper) have non-fixed, fold-away floors. Additionally, the convertible crate has horizontal side panels rising above sheep head height, whereas the standard crate has side panels with a top side rail that curves inwards (about 250 mm from the side) above the head of the sheep, designed to prevent them from jumping out of the crate. Therefore, there is slight reduction of head room on the standard crate (Figure 3). Only sheep in the upper deck of both vehicle types were analysed in this study.

Study A2: we compared between merino (M) and fat-tail (F) sheep breeds. Merino groups were subjectively identified as principally merino based on physical appearance. The fat-tail group consisted of Awassi, Damara or crossbred mixes of these breeds (Figure 4). Sheep were sourced from a live export pre-assembly feedlot where they had been in the feedlot between 3 and 5 days.

Study A3: we compared merino sheep transported from two points of origin (saleyard or feedlot). Animals sourced from the saleyard had been fasted anywhere between 24 and 72 h These animals were provided with water *ad libitum* and were held in point of origin groups. The animals had been transported between 1 and 2 days before the experimental transport trip and exposed to a new environment and drafted into pens on arrival. Animals sourced for the live export feedlot had feed available up until being brought into the loading yard (2–3 h prior to the transport event). Prior to transport, sheep were held at the feedlot between 3–5 days in the same groups in which they were transported.

Figure 4. View of a typical consignment of fat-tail sheep in a convertible crate.

2.3. Study B: Comparison of the Deck Level within a Crate

Study B: we compared between lower (L) and upper (U) decks for Standard (S) crates (Study B1: SU-SL), lower (L) and upper (U) decks for Convertible (C) crates (Study B2: CU-CL). A final analysis included all four combinations (Study B3). The sides of the upper and lower decks for both crate designs were similar, being composed of horizontal slatted metal. The upper decks for both crate designs had an open roof. Flooring of upper decks (non-slip metal grate over a solid metal sheet) differed according to crate type; the standard crate floor was fixed and the convertible crate floor was suspended, providing slightly less stability. Pens on the lower decks had solid metal sheeting roof.

Flooring of lower decks (non-slip metal grate over a solid metal sheet) also differed according to crate type; the convertible crate had a metal beam (150 × 100 mm W × H) that traversed the front pen, providing structural support for the convertible function of the vehicle (Figure 1). This beam required the sheep to stand astride it. The size and type of the flooring grate varied slightly between vehicles, as did the amount of compacted manure that was trapped between the floor and the grate.

2.4. Qualitative Behavioural Assessment

2.4.1. Selecting Footage for Scoring Clips

Continuous video footage was recorded during transport with a digital camera on both the upper deck (Panasonic SDR-H250, Belrose, NSW, Australia) and lower deck (GoPro Hero3 White Edition, and ContourRoam, Harvey Norman O'Connor WA, Australia) fixed to the railing of the trailer, just above sheep head height. From the footage approximately 1 min duration clips were edited out after the 10 min timepoint of the video so as not to introduce selection bias, as this was the period where the vehicle had commenced travelling at reasonable speed (on highway) and was predicted to be a time where behavioural responses of the sheep would be most marked. Clips were only rejected if the heads of the sheep were not clearly visible and subsequently taken at the next opportunity. Study A: footage was selected from approximately 10–15 min into the transport journey. Study B: clips were selected from upper and lower deck footage at the same time point at approximately 12 min after the truck departed the feedlot. Each clip was edited to highlight the focal groups by increasing the opacity of the surrounding animals in the same frame (Adobe Premiere Pro CS3 and Adobe After Effects CS3, Chatswood, NSW, Australia) and sound was removed thus obscuring evidence from the vehicle.

2.4.2. Observer Demographics

Observers were recruited from University staff and students and members of the public by advertising on notice boards and email and accepting all persons that responded. Observers were offered a supermarket voucher if they attended all sessions as compensation for their time. Before participating in the scoring of sheep footage, observers were asked to complete a questionnaire regarding their demographic background, experiences with sheep, as well as ranking their attitudes and opinions towards sheep and animal welfare (Supplementary Materials S1). The majority of observers (80%) were female, most (80%) worked with animals, and half had a high level of experience with sheep (Table 2).

Table 2. Demographic description of observers that contributed to QBA scoring.

Attribute	Category: # of Observers	
Study A (26 observers)		
Sex	Female: 21	Male: 5
Country of birth	Australia: 15	Other: 11
Residence	Urban: 24	Rural: 2
Area of study/employment: animal-related	Yes: 24	No: 2
Dietary preference: vegetarian	Yes: 4	No: 22
Purchasing habit: purchases own meat/eggs/dairy	Yes: 25	No: 1
Pet ownership	Yes: 22	No: 4
Level of experience with sheep	Low: 7; Medium: 4; High: 15	
Age (years)	<19: 1 20–29: 15 30–39: 8 40–49: 1 50–59: 0 60–69: 1 >70: 0	
Study B (26 observers)		
Sex	Female: 16	Male: 4
Country of birth	Australia: 11	Other: 9
Residence	Urban: 16	Rural: 4
Area of study/employment: animal-related	Yes: 13	No: 7
Dietary preference: vegetarian	Yes: 4	No: 16
Purchasing habit: purchases own meat/eggs/dairy	Yes: 20	No: 0
Pet ownership	Yes: 19	No: 1
Level of experience with sheep	Low: 7 Medium: 5 High: 8	
Age (years)	<19: 0 20–29: 8 30–39: 7 40–49: 2 50–59: 2 60–69: 0 >70: 1	

2.5. Viewing Sessions

Each observer was required to attend four sessions on campus or by correspondence (a term generation session and then three quantification sessions for each study). Observers (Study A; n = 26) attended sessions in November 2011 (treatment comparisons: Study A1, Study A2, and Study A3) and a second set of observers (n = 20; Study B) attended sessions in June 2013 (treatment comparisons: Study B1, Study B2 and Study B3). Observers were given detailed instructions on completing the QBA sessions but were not told about the experimental treatments or that the sheep were on a livestock vehicle.

For the term generation session, observers in both studies were shown 11 video clips of groups of sheep during road transport demonstrating a wide range of behavioural expression to allow observers to describe as many aspects of their expressive repertoire as possible. After watching each clip, observers were given 2 min to write down any words that they thought described that animal's behavioural expression. There was no limit imposed to the number of descriptive terms an observer could generate, but terms needed to describe not *what* the animal was doing (i.e., physical descriptions of the animal such as vocalising, chewing, tail flicking), but *how* the animal was doing it. Subsequent editing of the descriptive terms was carried out to remove terms that described actions, and terms that were in the negative form were transformed to the positive for ease of scoring (e.g., *unhappy* became *happy*). Each descriptive term was attached to a 100 mm visual analogue scale (min = 0 to max = 100). The list of terms was alphabetically arranged, therefore effectively randomly arranged and ensuring that terms with a similar meaning were not generally listed together.

For the quantification viewing sessions, observers viewed and scored video clips of animals under transport (clips were randomly arranged within each viewing session) using their own unique list of descriptive terms. Before session commencement, observers were given detailed instructions on how to score each animal's expression using the visual analogue scale: they were told to think of the distance between the zero-point and their mark on the scale as reflecting the intensity of the animal's expression. Observers viewed and scored 20 clips for each quantification session except for Study A2 where they viewed and scored 10 clips and for Study B3, where they viewed and scored 36 clips. For each clip, the score was to reflect the overall expression of all sheep visible.

2.6. Statistical Analyses

The distance from the start of the visual analogue scale to where the observer had made a mark was measured (mm) and these measurements were entered into individual observer Excel (Microsoft Excel 2003, North Ryde, NSW, Australia) files. These data were submitted to statistical analysis with Generalised Procrustes Analysis (GPA) as part of a specialised software package written for Françoise Wemelsfelder (Genstat 2008, *VS*.N International, Hemel Hempstead, Hertfordshire, UK; Wemelsfelder et al., 2000). Each Study (treatment comparison) was analysed separately, making a total of six independent analyses.

For a detailed description of GPA procedures, see Wemelsfelder et al. [12]. Briefly summarised, GPA calculates a consensus or 'best fit' profile between observer assessments through complex pattern matching. GPA provides a statistic (the Procrustes Statistic) which indicates the level of consensus (i.e., the percentage of variation explained between observers) that was achieved. The statistical process whereby this best-fit pattern, termed the consensus profile, is identified takes place independently of the meaning of individual terms used by observers. Whether this consensus is a significant feature of the data set, or, alternatively, an artefact of the Procrustean calculation procedures, is determined through a randomisation test [23]. This procedure rearranges at random each observer's scores and produces new permutated data matrices. By applying GPA to these permutated matrices, a 'randomised' profile is calculated. This procedure is repeated 100 times, providing a distribution of the Procrustes Statistic indicating how likely it is to find an observer consensus based on chance alone. Subsequently a one-way *t*-test is used to determine whether the actual observer consensus profile falls significantly outside the distribution of randomised profiles.

Through Principle Components Analysis (PCA), the number of dimensions of the consensus profile is reduced to several main dimensions (usually 2 or 3) explaining the variation between animals. GPA dimensions are interpreted by correlating the animals' scores to the observers' individual scoring patterns, producing individual observer word charts that describe the consensus dimensions through their association with each individual observer's terms. These word charts can then be compared for linguistic consistency. From these word charts, a list of terms describing the consensus dimensions was produced, by selecting terms for each observer that correlated strongly with those dimensions (i.e., the more highly correlated an individual term is with a dimension end, the more weight it has as a descriptor—positive or negative—for that dimension). Each video clip of animals receives a quantitative score on each of these dimensions, so that the transport trip's position in the consensus profile can be graphically represented in two- or three-dimensional plots. Each plot represents each of the transport trips in the respective treatments, where the position of the transport trip indicates its scores on each GPA axis.

To investigate treatment effects, the observer scores for each GPA dimension (response variables) were analysed using mixed-model ANOVAs, with driver ID and observer ID included as random factors to account for driver behaviour/truck design differences and repeated observations. For Study B3, we included both crate and deck as two independent factors. Analyses were carried out using Statistica software, version 9 (StatSoft-Inc, Tulsa, OK, USA).

2.7. Survey of Drivers

To gain preliminary information about drivers and procedures involved with commercial livestock carriers, two short surveys were carried out while we were collecting footage (Spring 2011). Each driver was given a questionnaire (Supplementary Materials S2) to determine driver background and their opinions of the livestock transport industry. Information on each journey was gathered using a questionnaire that was completed both through viewing the loading process and questioning the driver (Supplementary Materials S3).

3. Results

The 26 observers participating in Study A generated a total of 115 unique terms to describe the behavioural expression of the sheep viewed in the term generation session, with an average of 17 ± 6 (min: 6, max: 31) terms per observer. The 20 observers participating in study B generated a total of 85 unique terms, with an average of 12 ± 4 (min: 6, max: 21) terms per observer. There was significant consensus between observer scores for each of the six treatment comparisons; Procrustes Statistics are shown in Table 3. Terms with the strongest correlation with each of the GPA dimensions for each treatment comparison are shown in Table 3 (max 10 terms); the first two terms are used as labels for each GPA dimension (Figures 5–7).

We recorded significant effects of vehicle driver (included as a random factor in all our analyses) on GPA dimension scores for all except Study B3 (Table 3).

3.1. Study A: Comparison of 1. Trailer Crate Design, 2. Sheep Breed and 3. Point of Origin

3.1.1. Study A1 Trailer Crate Design: Standard Crate vs. Convertible Crate

The first three GPA dimensions explained a total of 66.5% of the variation between animals (Table 3). The positions of individual sheep on the first two GPA dimensions are shown in Figure 5a. There was a significant treatment effect for GPA dimension 1 ($p < 0.001$) with sheep transported in a standard crate scored as more *calm*/*relaxed* than sheep transported in a convertible crate, which were scored as more *agitated*/*anxious*. There were no significant treatment effects on GPA dimensions 2 or 3. Of the transport trips assessed, 19 of them stocked adult sheep. One transport journey that stocked lambs; this transport journey is labelled 'C2' in Figure 5a and stood out as an outlier, with sheep in this clip scored as more agitated/anxious than any other clip.

3.1.2. Study A2 Sheep Breed: Merino vs. Fat-Tail Sheep

The first three GPA dimensions explained a total of 65.0% of the variation between animals (Table 3). The positions of individual sheep on the first two GPA dimensions are shown in Figure 5b. There was a significant treatment effect on GPA dimensions 1 ($p = 0.006$) and 2 ($p = 0.011$) with fat-tail sheep scored as more *agitated*/*distressed* (GPA1) and *curious*/*alert* (GPA2) than merino sheep, which were scored as more *calm*/*relaxed* and *tired*/*content*. There were no significant treatment effects on GPA dimension 3.

3.1.3. Study A3 Point of Origin: Feedlot vs. Saleyard

The first three GPA dimensions explained a total of 63.1% of the variation between animals (Table 3). There was a significant treatment effect on GPA dimension 3 ($p = 0.014$), with sheep transported from the feedlot scored as more *comfortable*/*relaxed* than sheep transported from the saleyard, which were more *nervous*/*curious*. However this dimension only explained a small proportion (5.8%) of the variability in data. There were no significant differences in GPA dimension 1 and 2 scores.

3.2. Study B: Comparison of the Deck Level within a Crate

3.2.1. Study B1 Deck Level: Standard Crate Upper vs. Lower Deck

The first three GPA dimensions explained a total of 67.6% of the variation between animals (Table 3). The positions of individual sheep on the first two GPA dimensions are shown in Figure 6a. There was a significant treatment effect for GPA dimension 2 ($p < 0.001$) and 3 ($p < 0.001$), with sheep transported in the upper deck scored as significantly more *alert*/*curious* and *stressed*/*alert* than sheep transported in the lower deck, which were scored as more *miserable/unsure* and *nervous/agitated*. There were no significant treatment effects on GPA dimension 1.

3.2.2. Study B2 Deck Level: Convertible Crate Upper vs. Lower Deck

The first three GPA dimensions explained a total of 72.4% of the variation between animals (Table 3). The positions of individual sheep on the three GPA dimensions are shown in Figure 7a,b. There were significant treatment effects for all three GPA dimensions, with sheep on the upper deck scored as significantly more *calm/relaxed*, *alert/curious*, and *agitated*, *cramped* than sheep on the lower deck, which were scored as more *agitated/stressed*, *dejected/weary* and *nervous/worried*.

3.2.3. Study B3 Standard versus Convertible Crate: Upper and Lower Deck

The first three GPA dimensions explained a total of 71.1% of the variation between animals (Table 3). The positions of individual sheep on the first two GPA dimensions are shown in in Figure 6b. There was no significant effect of crate on the GPA dimension scores. There was a significant effect of deck for GPA dimension 2 (p = 0.008) with sheep on the upper decks during road transport scored as more *curious/alert* than sheep on the lower decks, which were scored as more *distressed/stressed*. The crate x deck interaction terms were not significant.

3.3. Driver Questionnaire

Fourteen drivers that participated in the study were asked what they believed should be the most important priorities for improvement of the livestock transport industry. Their responses were;

- Five drivers believed that the industry required better enforcement of withholding (curfew) periods.
- Drivers expressed concern about the waiting time at the wharf prior to sheep being loaded for export.
- Three drivers believed that loading density is an important priority; in particular, the density should be slightly lower, especially on long distance trips.
- Two drivers stated that education was required for producers to better prepare animals for transport and therefore improve loading and reduce transport stress and to understand the benefits of lower densities. Such preparation could include habituation to loading ramps, handling and humans, and education on the design of loading facilities and minimum requirements (e.g., a raised loading ramp).
- Two drivers identified that they required better support in refusing to load animals that they believed were in poor condition or not fit to load.

In response to being asked what drivers believed were the most important animal welfare issues that needed to be addressed during road transport, the following was indicated:

- condition of animal and impact of condition on stress and welfare during transport;
- having the right to refuse an emaciated or pregnant animal, guidelines for stocking rate during transport;
- rest yards at east-west state border with feed and water available; and
- driver education for handling sheep during loading and unloading.

In response to being asked what specific behaviour drivers thought would indicate that a sheep's welfare was compromised during transport, the following was indicated:

- body position: lying down, in corner of crate or underneath other sheep, legs out of crate;
- movements such as limping or tucked up when loading or unloading;
- breathing style: reparation distress, tongue out when panting;
- general demeanour: head down, position of eye; and
- vocalisation.

Table 3. Terms used by observers to describe sheep behavioural expression during road transport. Terms showing the strongest correlations ($r > 0.5$) with low and high values on each Generalised Procrustes Analysis (GPA) dimension of the consensus profile (maximum 10 terms presented). Order of terms is determined firstly by number of observers to use that term (in parentheses where >1) and secondly by weighting of each term.

Treatment, Procrustes Statistic †	GPA Dimension	Low Values	High Values	Treatment Effect
Study A				
A1: S-C PS: 56.64% (t_{99} = 43.8, $p < 0.001$)	1 (53.2%)	Agitated (7), anxious (5), nervous (5), worried (5), concerned (3), scared (3), jumpy (2), fidgety (2), alert (2), distressed (2)	Calm (11), relaxed (7), settled (3), restful (2), comfortable (2), subdued, happy, tolerant, accepting, bored	Crate: $F_{1, 6} = 48.85$, $p < 0.001$ Sheep in a standard crate scored higher than sheep in a convertible crate Driver ID (random factor): $F_{6, 487} = 5.90$, $p < 0.001$
	2 (8.5%)	Curious (7), alert (3), inquisitive (2), nervous (2), interested, confident, comfortable, puzzled, at ease, watchful	Frightened, frustrated, agitated, bored, annoyed, distressed, anxious, bossy	Crate: $F_{1, 6} = 0.29$, $p = 0.609$ Driver ID (random factor): $F_{6, 487} = 26.84$, $p < 0.001$
	3 (4.8%)	Tired (2), apprehensive, struggling, pissed off, sad, fidgety, certain, alert, sleepy, bored	Confident, relaxed, sleepy, depressed, settled, satisfied	Crate: $F_{1, 6} = 0.96$, $p = 0.367$ Driver ID (random factor): $F_{6, 487} = 6.67$, $p < 0.001$
A2: M-FT PS: 56.11% (t_{99} = 12.01, $p < 0.001$)	1 (42.5%)	Agitated (6), distressed (6), nervous (5), anxious (4), jumpy (3), pushy (2), alert (2), wary (2), frustrated (2), fidgety (2)	Calm (6), relaxed (4), comfortable (4), happy (3), settled (2), patient (2), composed (2), restful, mellow, unphased	Breed: $F_{1, 4} = 27.98$, $p = 0.006$ Merino sheep scored higher than fat-tail sheep Driver ID (random factor): $F_{2, 223} = 30.87$, $p < 0.001$
	2 (12.6%)	Curious (5), alert (4), inquisitive (4), worried (3), interested (2), comfortable (2), nervous (2), attentive, aware, confident	Tired (2), content (2), happy, calm, scared, settled, annoyed, anxious, frustrated, nervous	Breed: $F_{1, 14} = 8.44$, $p = 0.011$ Merino sheep scored lower than fat-tail sheep Driver ID (random factor): $F_{2, 223} = 7.30$, $p = 0.001$
	3 (9.9%)	Nervous (3), alert (3), interested (2), curious (2), annoyed (2), calm (2), jumpy, nonchalant, anticipating, restless	Relaxed (3), tired (2), alert (2), anxious (2), scared (2), comfortable (2), sleepy (2), unsure, insecure, resigned	Breed: $F_{1, 3} = 0.05$, $p = 0.837$ Driver ID (random factor): $F_{2, 223} = 75.96$, $p < 0.001$
A3: FL-SY PS: 49.2% (t_{99} = 29.051, $p < 0.001$)	1 (46.6%)	Agitated (4), anxious (4), frightened (3), worried (3), nervous (3), panicked (3), scared (2), restless (2), jumpy, fretful	Calm (6), bored (3), relaxed, casual, accepting, placid, comfortable, settled, patient, composed	Origin: $F_{1, 6} = 0.12$, $p = 0.745$ Driver ID (random factor): $F_{5, 469}$=27.51, $p < 0.001$
	2 (10.7%)	Curious (3), happy (2), alert (2), bored (2), interested (2), comfortable, calm, settled, resigned, content	Curious (2), agitated (2), resigned, interested, tired, lethargic, depressed, struggling, distressed, bored	Origin: $F_{1, 18} = 1.12$, $p = 0.304$ Driver ID (random factor): $F_{5, 469}$=4.21, $p < 0.001$
	3 (5.8%)	Nervous (2), curious (2), distressed, excited, interested, agitated, annoyed, alert	Comfortable (2), relaxed (2), aware, stressed, mellow, calm, settled, confident, happy, agitated	Origin: $F_{1, 24} = 7.04$, $p = 0.014$ Sheep from feedlot scored higher than sheep from saleyard Driver ID (random factor): $F_{5, 469} = 3.11$, $p = 0.009$

Table 3. *Cont.*

Treatment, Procrustes Statistic †	GPA Dimension	Low Values	High Values	Treatment Effect
Study B				
B1: SU-SL PS: 42.75% (t_{99} = 16.45, $p < 0.001$)	1 (35.9%)	Agitated (7), stressed (4), pushy (3), distressed (3), worried (3), uneasy (2), restless (2), nervous (2), scared(2), distressed (2)	Calm (7), comfortable (4), relaxed (2), happy (2), placid, quiet, accepting	Deck (S): $F_{1, 377} = 0.41$, $p = 0.521$ Driver ID (random factor): $F_{2, 377} = 15.03$, $p < 0.001$
	2 (20.9%)	Miserable, unsure, tired, restful	Alert (9), curious (6), interested (5), happy (3), inquisitive (2), watchful (2), comfortable (2), observant, confused, confident	Deck (S): $F_{1, 377} = 136.22$, $p < 0.001$ Sheep transported in the upper deck scored higher than sheep transported in the lower deck Driver ID (random factor): $F_{2, 377} = 6.04$, $p = 0.003$
	3 (10.8%)	Nervous (2), agitated (2), stressed, distressed, settled, weary, wary, dejected, collected, confused	Stressed (2), alert (2), restless (2), distressed, adjusting, anxious, quiet, fidgety, unsupported, relaxed	Deck (S): $F_{1, 377} = 25.40$, $p < 0.001$ Sheep transported in the upper deck scored higher than sheep transported in the lower deck Driver ID (random factor): $F_{2, 377} = 4.21$, $p = 0.015$
B2: CU-CL PS: 45.34% (t_{99} = 23.67, $p < 0.001$)	1 (56.1%)	Agitated (6), stressed (5), nervous (4), frightened (4), restless (4), distressed (3), uneasy (3), anxious (2), unsettled (2), worried (2)	Calm (9), relaxed (7), comfortable (6), happy (3), quiet (2), resolved, settled, at ease, resigned, restful	Deck (C): $F_{1, 376} = 22.54$, $p < 0.001$ Sheep transported in the upper deck scored higher than sheep transported in the lower deck Driver ID (random factor): $F_{3, 376} = 32.58$, $p < 0.001$
	2 (8.5%)	Dejected, weary, agitated, tired, scared	Alert (6), curious (4), interested (2), confident (2), relaxed (2), happy, wary, observant, aware	Deck (C): $F_{1, 376} = 22.54$, $p < 0.001$ Sheep transported in the upper deck scored higher than sheep transported in the lower deck Driver ID (random factor): $F_{3, 376} = 32.58$, $p < 0.001$
	3 (7.8%)	Nervous, worried, unsure	Agitated (2), cramped (2), curious (2), alert, aware, interested, relaxed, squashed	Deck (C): $F_{1, 376} = 49.17$, $p < 0.001$ Sheep transported in the upper deck scored higher than sheep transported in the lower deck Driver ID (random factor): $F_{3, 376} = 24.54$, $p < 0.001$
B3: SU-SL-CU-CL PS: 36.86% (t_{99} = 32.699, $p < 0.001$)	1 (43.2%)	Agitated (5), nervous (3), restless (3), uneasy (3), frightened (3), anxious (2), stressed (2), worried (2), distressed (2), confused (2)	Calm (7), relaxed (5), comfortable (4), happy, at ease, settled	Crate: $F_{1, 5} = 0.35$, $p = 0.581$ Deck: $F_{1, 6} = 0.01$, $p = 0.968$ Crate x deck: $F_{1, 5} = 0.63$, $p = 0.466$ Driver ID (random factor): $F_{5, 5} = 4.30$, $p = 0.065$
	2 (19.1%)	Distressed (2), stressed (2), bored, scared, freaked, miserable, quiet, unsure, worried, sad	Curious (8), alert (7), aware (3), interested (2), observant (2), confident, happy, relaxed	Crate: $F_{1, 6} = 0.47$, $p = 0.525$ Deck: $F_{1, 8} = 12.35$, $p = 0.008$ Sheep transported in the upper deck scored higher than sheep transported in the lower deck Crate x deck: $F_{1, 5} = 0.51$, $p = 0.509$ Driver ID (random factor): $F_{5, 5} = 1.42$, $p = 0.355$
	3 (8.8%)	Nervous (2), curious (2), distressed, excited, interested, agitated, annoyed, alert	Quiet (2), collected, calm, confused, accepting, aloof, peaceful	Crate: $F_{1, 5} < 0.01$, $p = 0.994$ Deck: $F_{1, 4} = 4.50$, $p = 0.114$ Crate x deck: $F_{1, 4} = 0.82$, $p = 0.412$ Driver ID (random factor): $F_{5, 5} = 2.02$, $p = 0.222$

† PS: Procrustes Statistic showing the percentage of variance in the dataset that could be attributed to the consensus in scoring between individual observers and the results for the one-way *t*-test comparing this result with a mean randomised profile of the same dataset, indicating that the consensus between observers in their use of descriptive terms to quantify the behavioural expression of these sheep was statistically significant.

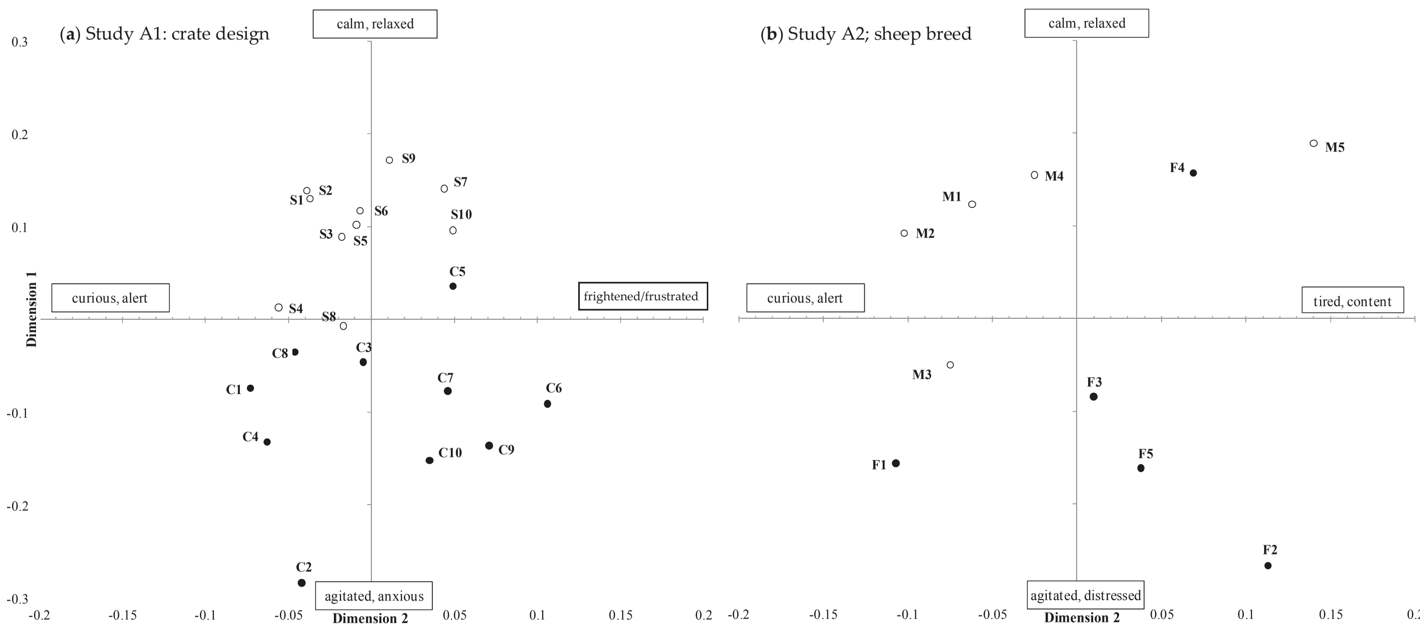

Figure 5. Positions of individual transport clips (represented by numbers) on Generalised Procrustes Analysis (GPA) dimensions 1 (y axes) and 2 (x axes). (**a**) Study A1: crate design experiment (S: standard crate, open circles, or C: convertible crate, closed circles); (**b**) Study A2: sheep type experiment (M: Merino sheep, open circles, or F: Fat-tail sheep, closed circles). Bold text indicates GPA dimensions 1 for both studies were statistically significantly different between treatments.

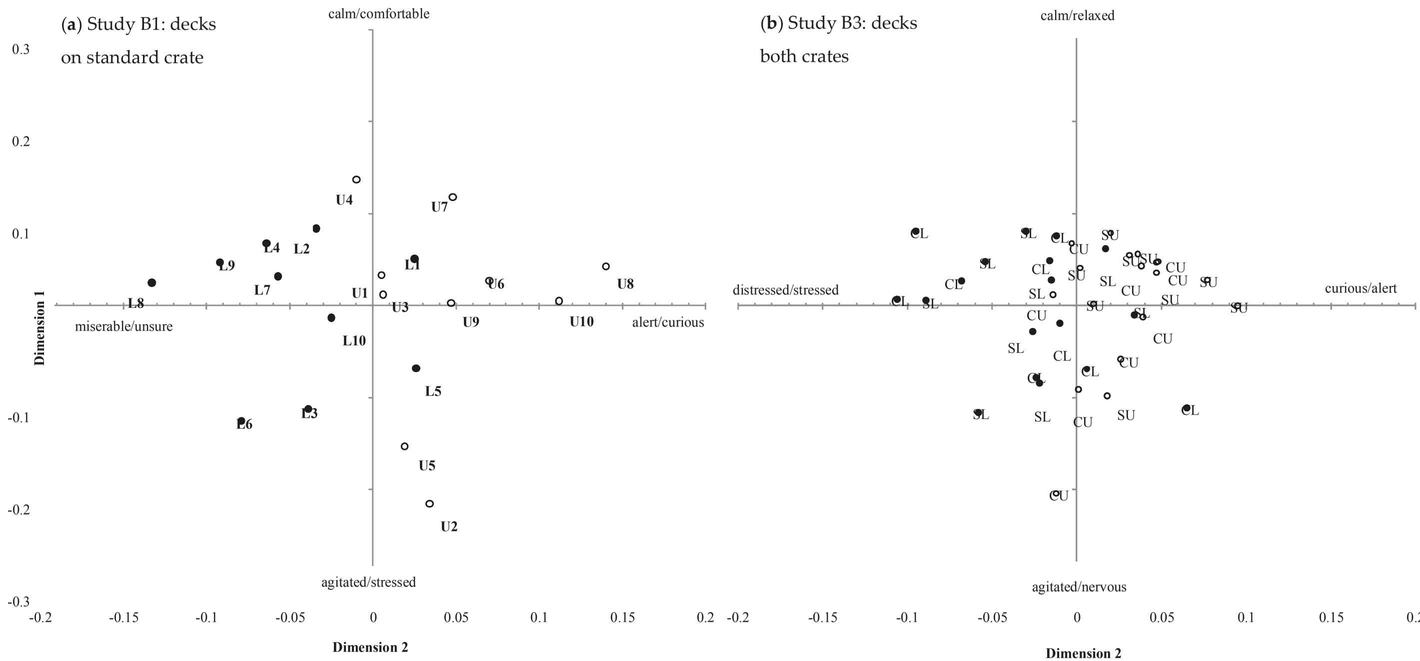

Figure 6. Positions of individual transport clips on GPA dimensions 1 and 2 obtained for (**a**) Study B1 standard crate experiment (Numbers represent each transport trip, U: upper deck, open circles, or L: lower deck, closed circles) and (**b**) Study B3 crate design experiment (SU: standard crate upper deck, open circles, SL: standard crate lower deck, closed circles, CU: convertible crate upper deck, open circles and CL: convertible crate lower deck, closed circles). Bold text indicates GPA dimensions 2 for both studies were statistically significantly different between treatments.

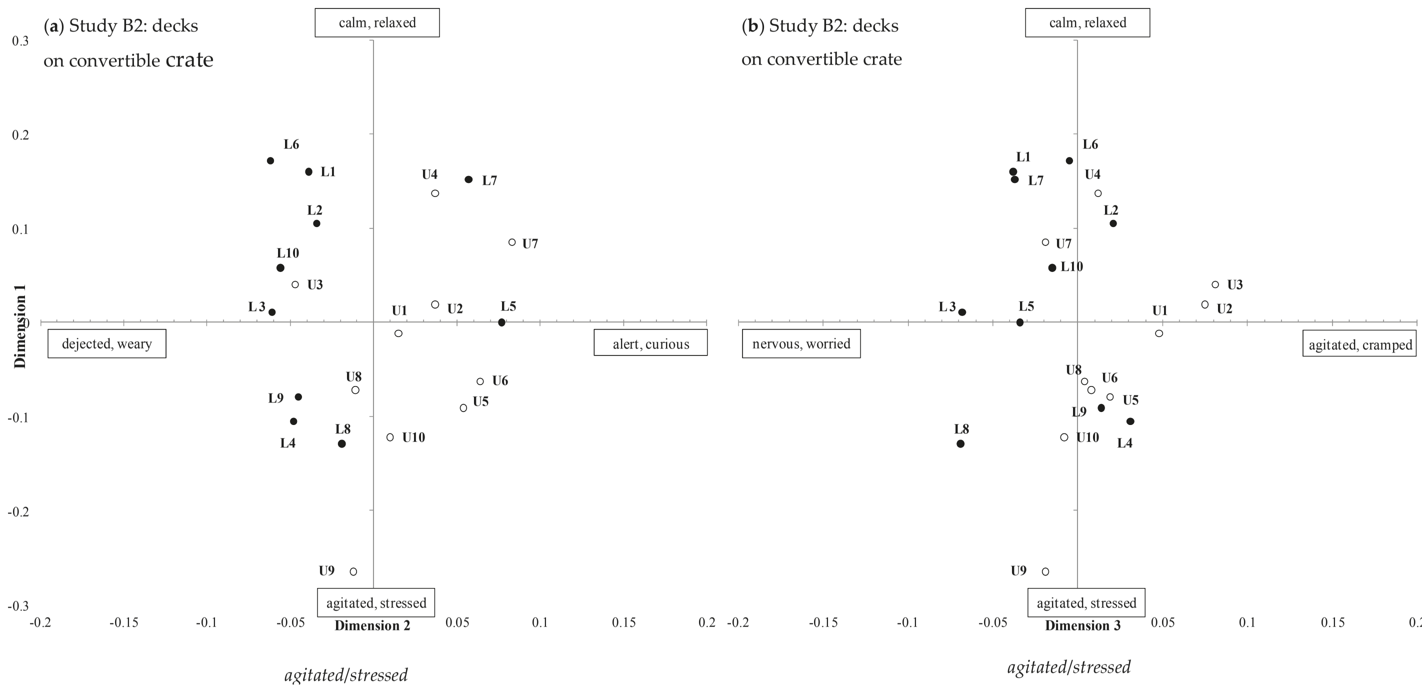

Figure 7. Positions of individual transport clips (numbers represent each transport trip) obtained from Qualitative Behavioural Assessment in Study B2 for convertible crate experiment (U: upper deck, open circles, or L: lower deck, closed circles) showing the same data plotted for (**a**) GPA dimensions 1 and 2 and (**b**) GPA dimensions 1 and 3.

4. Discussion

Using Qualitative Behavioural Assessment, observers from a range of backgrounds and with different levels of sheep experience distinguished behavioural expression of sheep subjected to different physical conditions during commercial transport (e.g., trailer crate type and deck level), between sheep of different breeds and even between sheep transported from a livestock saleyard compared with those from a feedlot. Our previous studies under experimental transport conditions showed behavioural expression reflected changes in the underlying physiological state of the livestock that were typically associated with the stress response [15–18]. Such behavioural responses are likely to predict the wellbeing of the animals, and as indicators of welfare they may be associated with production traits [24,25]. Importantly, the early identification of animal responses to husbandry procedures (e.g., farm/facility/transport setting) allows the modification of procedures or facilities in a way that optimises the health and welfare of animals [26,27]. Even subtle differences in demeanour can therefore be informative.

4.1. Study A1: Trailer Crate Design

The observers detected differences in merino sheep travelling in the upper deck of two types of trailer crates over the same road journey. Sheep in the convertible crate were scored as more *agitated/anxious* than sheep in the standard crate, which were more *calm/relaxed*. Hence, this dimension corresponds to a distinction between emotional valence of sheep, with similar terms used to those in our previous studies using a small, single-deck trailer [15,16]. Prior to this experiment, sheep had been handled in a similar way; they were yarded 2–3 h before loading, had minimal food curfew and were moved through the same loading facility.

Although the two crate designs had similar loading density, the larger pen size in the convertible crate allowed the animals the freedom to pack tightly together in the corners, leaving others more room to move. This may have allowed more physical movement and less sheep-to-sheep support within the whole pen; increased space availability has been demonstrated to increase movement in lambs, but also increase slipping and carcass bruising [28,29]. Structural differences in the sides and the floor of the upper decks of the two crates may have influenced how sheep travelled. The slight reduction of head room caused by the side rails on the standard crate may decrease physical movement or create a more confined feeling for the sheep. The floor of the upper deck of the convertible crate is less stable as it allows greater suspension and vibration than that of the standard crate. One possible explanation is that this increased flexibility of the floor may lead to sheep experiencing less secure footing.

4.2. Study A2: Sheep Breed

Observers detected differences in merino and fat-tail sheep travelling in the same type of trailer crate (a standard crate) over the same road journey. Merino sheep were scored as more *calm/relaxed* and *curious/alert* compared with the Fat-tail sheep on the same vehicle and route, which were scored as more *agitated/distressed* and *tired/content*. These dimensions appear to reflect activity and/or arousal. However, given that we only had five replicate transport events for each sheep breed group, our data warrants following up with additional studies on how different sheep breeds deal with road transport.

Prior to this experiment, sheep had been handled in a similar way (yarded 2–3 h earlier, with no feed curfew and moved through the same loading facility). Thus, the behavioural response to transport appeared to be linked to breed. The behavioural responses may reflect the ability of the sheep to cope with the transport stressors and this may be linked to their state of fearfulness. Previous studies have demonstrated physiological differences (plasma cortisol, packed cell volume) in sheep genotypes, with some more reactive to land transport than others [30]. In a non-transport example, Miller et al. [31] demonstrated that QBA scores were different for feral goats exposed to varying levels of human interaction, suggesting that the scores reflected habituation and possibly their ability to adapt to the challenge of confinement. Grandin [32] found that animals with an excitable temperament may have greater difficulty adapting to a situation, while calmer animals may adapt more easily and become less stressed. Identification and selection of breeds of sheep that better cope with the transport environment represents one way to improve the welfare of livestock.

4.3. Study A3: Point of Origin

We only found subtle differences in the demeanour of sheep that originated from either a saleyard or feedlot—the only differences were on GPA dimension 3, which only accounted for 5.8% of the total variation in observer scores. Merino sheep being transported from a saleyard were more *nervous/curious* than those transported from a feedlot, which were more *comfortable/relaxed*. We had expected that there would be differences in demeanour for these animals because sheep had been exposed to very different experiences over the preceding 24–48 h. Prior to yarding for 2–3 h before loading, sheep from the feedlot had been fed in a pre-export feedlot for approximately 5 days. These sheep were penned in large flocks and most likely habituated to the feed, shed and daily routine; they also had minimal time off food and water prior to transport. By contrast, sheep loaded at the saleyard had experienced a novel, noisy and high arousal environment over the previous 1–3 days. Potential stressors for saleyard sheep include a transport journey from their farm of origin, feed curfew of 24–72 h, separation and mixing of social groups, handling through several small yards by sheep dogs, as well being confronted by the presence of humans at close proximity. The subtle differences in demeanour could indicate that the transport environment is sufficiently novel and challenging to mostly overshadow prior affective condition. Further studies comparing more diverse backgrounds, such as from farm of origin and comparing varied lengths of time in confinement or curfews could provide further insight into the effect of point of origin.

4.4. Study B: Deck Level

We recorded a significant difference in the demeanour of sheep positioned in the upper and lower decks on for both vehicle crate types, with sheep on the upper deck scored as more *alert/curious* and *agitated/cramped* than those on the lower deck, which were scored as more *miserable/unsure*, *dejected/weary*, *nervous/worried* and *distressed/stressed*. The final study that allowed observers to compare sheep expression directly from journeys from all four positions, that is standard upper and lower and convertible upper and lower, confirmed the significant difference was detected between deck levels but not crate type, indicating that effect of deck level overshadowed any difference between crate design. Interestingly, the term *nervous* was highly loaded on the low arm of the GPA3 for all but one analyses but the term *cramped* was only loaded on GPA3 analysis of CU-CL. Although the third dimension accounts for a small percentage of variation, it seems sheep in the upper deck were viewed as more cramped than the lower.

How important this deck position is to sheep is unknown and may be influenced by other factors such as weather and climate. Sheep positioned on the upper deck are likely to have more visual and tactile stimulation as they experience wind, sun or rain, which may affect how they experience the journey whereas those in lower decks are largely screened from such exposure. It has been demonstrated that many novel aspects associated with transportation, apart from the vehicle movement itself, can trigger behavioural responses in sheep [33]. The behavioural dimensions identified in this study concur with key qualitative descriptors for sheep demeanor that were identified in previous studies, such as *agitated*, *distressed*, *alert* and *curious* [13,15,16,34]. These dimensions used terms semantically consistent with a valence of 'mood' (GPA 1) and 'arousal' (GPA2) that have been similarly reported elsewhere [13,27,31,34].

4.5. Limitations of This Study

Because we were working under commercial conditions and made every attempt to not interfere with normal transport processes, we had relatively little control over the conditions that animals were transported under. For example, we had only few transport events for fat-tail sheep that were transported under matched conditions as merino sheep, which restricted our sample size for this treatment comparison. Furthermore, in order to restrict the number of variables between transport events, clips were taken from routine transport events that matched each other in as many ways as possible.

The convertible crate design is relatively new, and only a few livestock trucks of such design in Western Australia. Multiple transport events on specific vehicles (driven by its owner/driver) were therefore not strictly independent. We tested for such an effect in our data (driver included as a random factor) and identified significant effects of vehicle driver on the demeanour of sheep, which could reflect either their driving style or the specific design of their vehicle.

Finally, although we attempted to obscure evidence of the animals being on a truck by editing our video clips, we cannot guarantee that observers were unaware of the treatment difference for our deck level experiment. Obtaining footage from the lower decks with adequate lighting and adequate visualisation of groups of sheep with limited head room was challenging. The contrast with sheep in sunshine on the upper deck could not be avoided. Such cues are likely to influence how observers score footage because QBA scoring is sensitive to environmental clues which can introduce undesirable bias due to the observers' judgment of that context [35,36]. We used different cameras to account for different lighting levels on the different decks and therefore the quality of the footage was equitable, but animals on the lower decks are more obviously in a confined place than those on the top deck.

4.6. Driver Questionnaire

We found significant effects of driver ID (included as a random factor in our analyses) on the GPA dimension scores (for all but Study B3), which suggested that individual driver behaviour or truck design influenced the behaviour of independent groups of sheep transported in their trucks. Cockram et al. [6] found a significant effect of driver (comparing two drivers) on losses of balance and disturbance in sheep transported by truck, with ~80% of losses of balance caused by driving events such as acceleration, braking, cornering and uneven road surfaces. Awareness of driving style can help improve driving style to minimize stress to the animals, and Grandin [20,37] showed that there was an increase in the welfare of animals when livestock drivers received a bonus payment for meat quality of the animals transported. On the other hand, truck design is also likely to influence animal welfare during transport [38]. For example, Huertas et al. [29] found differences in carcass bruising with different truck designs. We could not distinguish between driver and truck in our study—this would require an experimental design where multiple drivers used the same truck which was not within the scope of our study.

The short driver survey allowed researchers to informally discuss the concerns of a small number of drivers directly responsible for the care of transported animals. Some areas for improvement identified, including handling, preparation of sheep before transport (length of curfew) and long-distance journeys. Importantly, drivers were able to describe sheep behaviour that alerted them to situations of poor animal welfare. These surveys allowed the initiation of dialogue about the shared responsibility of ensuring livestock were well cared for during land transport. A willingness to participate in future research and monitoring was a useful outcome from such conversations. Further engagement of industry at all levels with research is required to ensure continued positive changes to livestock transport. Importantly, the drivers did not volunteer driving style or truck design as an issue of welfare concern. In informal discussions with individual drivers, they raised their observations about differences between deck levels, although they did not identify this as an animal welfare issue in their formal surveys.

5. Conclusions

Observers showed strong agreement as to how the sheep were rated, supporting the use of human observers to score sheep behaviour during commercial transport, with minimum intrusion into the normal procedures of animal handling. QBA is a valuable method for assessing the wellbeing of sheep in production systems [14]. Observer rating of animal behaviour offers many advantages, not the least that it is practical and relatively inexpensive. The method is also repeatable, reliable and relevant to animal welfare assessment.

The livestock transport process is complex and involves handling of animals at multiple facilities by different groups of stockpersons working under time constraints. Hence a non-invasive assessment using video recording may prove an efficient and effective means to measure the animals' responses to handling and movement and offers insight into how well livestock are coping in what are stressful, but necessary, environments. The inclusion of a qualitative measure adds an important interpretative element to any welfare analysis or assurance scheme. With further validation at specific points within the livestock production chain, QBA appears as a promising welfare-relevant monitoring tool for industry.

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animals

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Communication

Effects of Short Transport and Prolonged Fasting in Beef Calves

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Simple Summary: Marketing is inherently stressful for animals because they are removed from their home environment, handled, and transported. When sold at a livestock market, the events associated with transport are duplicated, in that animals are delivered to and then transported from the market, animals are kept confined in an unknown environment and are often mixed with unfamiliar animals, and fasting times increase. For calves, the stress of weaning is added, because the weaning process often takes place moments before being loaded for transport. In Chile, approximately one million cattle go through livestock markets annually and over 30% of them, being the largest category, are calves. Some studies have shown that calves sold through markets suffer from extended fasting periods, even when exposed to only short transportation times. The aim of this study was to determine the consequences of a short transportation time followed by an extended period without food and water. This was undertaken by measuring variables related to stress in beef calves. The results obtained showed a significant physiological effect on body temperature, blood indicators and live weight (LW). Calves lost a mean of 10 kg each after 24 h of fasting. LW loss is probably the most significant economic effect, since animals are traded based on weight. Further studies to measure the impact during true, commercial marketing are needed.

Abstract: Marketing is a stressful process for beef calves, because they are removed from their environment, often weaned just before loading, loaded, transported, and unloaded. It also involves extended periods without food and water and mixing with unfamiliar animals in an unknown environment. Some studies have shown that calves sold through markets are exposed to extended fasting periods even when they undergo only short transportation times. The aim of this controlled study was to determine the consequences for beef calves of a short transportation time followed by a prolonged time without food and water on their tympanic temperature (TT), maximum eye temperature (MET), blood variables related to stress, and live weight. Ten calves were transported for 3 h and then kept in an outdoor pen for 21 h, completing a 24 h fasting period. Sampling took place before loading, after transport and unloading, and then after completing 24 h without food and water. TT, MET, blood glucose, and creatine kinase (CK) increased significantly after transportation. Live weight decreased across sample times (mean of 10 kg per calf after 24 h of fasting), which was consistent with the higher concentration of β-HB found after fasting. Further studies to measure the actual consequences of true, commercial marketing on calf welfare and productivity are needed.

Keywords: beef calves; transport; fasting; marketing

1. Introduction

Selling cattle through livestock markets is still common in many South American countries [1,2]. At livestock markets, animals are handled by anonymous, and generally untrained, handlers [3], and are exposed to at least twice as many physical and psychological stressors than calves sold directly from farm to farm [4]. In Chile, approximately one million cattle go through livestock markets annually and over 30% of them, being the main category sold, are calves. Preliminary results [5] show that the mean transport time of calves from origin (farm) to the market is only 75 min (although it ranged between 5 min and 13 h) and from the market to the final destination (usually another farm where calves are fattened) is only 45 min (5 min–40.5 h). However, including the time spent in the holding pens (without any food or water), calves generally underwent at least 12 h of fasting, and frequently up to 24 h of fasting, which is the maximum time allowed by Chilean legislation [6,7].

Although in many countries there are now regulations regarding the transport and slaughter of livestock [1], fasting times remain long in many South American countries for diverse reasons [2]. The short- and long-term effects of transport and fasting on welfare and production indicators in calves have not attracted as much attention as they have in slaughter weight cattle, and they are also more difficult to measure. Werner et al. [8] described body weight changes and some blood constituent changes related to the stress response during a 63-h transport of recently weaned calves (approximately 240 kg) in the Chilean Patagonia. The high cortisol concentration values before transport, found by Werner et al. [8], suggest that the handling processes before transport (herding from distant fields and regrouping in pens before loading and weaning), which are common practice in extensive systems in the Patagonia, were already stressful for the calves, and represented the highest mean value found throughout all sampling stages. In the same study [8], there was significant body weight loss in the calves after the 63-h period (14% of LW, live weight). A recovery period of 30 days was required, probably because the calves ate and drank less than usual in the new environment but continued to mobilize body reserves. These results show that the long-distance transport of calves not only has an effect on animal welfare, but also creates economic losses for producers.

The marketing process is inherently stressful for calves because they are taken away from their environment, often weaned just before loading, loaded, unloaded, and transported. When selling through a livestock market additional stress can be caused because: the events associated to transport happen more than once, as they go to and from the market; animals are kept confined in an unknown environment and are often mixed with unfamiliar animals; and time without food and water increases. Physiological indicators commonly used to measure stress in animals have been reviewed by Knowles et al. [9]. Blood variables as indicators of stress have some limitations given that the handling and restraining of animals is required during sampling, which in turn produces a stress response [10].

When an animal becomes stressed, the hypothalamic–pituitary–adrenocortical (HPA) axis is activated because of the increase in catecholamines and cortisol concentrations, in addition to blood flow responses, and will produce changes in heat production and heat loss from the animal [11]. During a physical attack, or in response to a painful stimulus, blood can be diverted from the cutaneous bed and redirected to bodily organs with more urgent metabolic requirements [12]. The effect of this vasoconstriction is a decrease in skin temperature, which can be detected by infrared thermography (IRT). IRT measures the superficial temperature of objects in a non-invasive manner and has been widely used to measure the superficial body temperature in animals [13–16]. Eye temperature has been shown to be a more consistent measure of temperature change than other anatomical areas, particularly in response to stress, and is not interfered with by fur or hair [17–23].

This is a preliminary study with the aim of determining the consequences for beef calves of a short transportation time followed by a prolonged time without food and water on their tympanic temperature (TT), maximum eye temperature (MET) measured using IRT, blood variables related to stress, and live weight.

2. Materials and Methods

The Bioethics Committee "Use of animals in research" of the Universidad Austral de Chile, approved the present study (Application N°325/2018).

Ten black and red Angus calves, mixed male and female, with a mean live weight of 146.1 ± 19.1 kg, weaned a month before the experiment, and clinically healthy were used. All calves had been bred on the same farm where the experiment was performed; they were kept on pasture during the day, with water *ad libitum*, and were put in a barn overnight, receiving 1 kg/head of sugar beet pulp and hay.

The study was carried out in Lanco, Chile, during winter. The study started at 9 a.m. and the calves were calmly moved approximately 50 m from the barn to a pen, and then to a race and chute that had a head-holder for immobilization, blood sampling, and measurement of other variables. Sampling took place before loading, after transport and unloading, and again after 24 h without food and water in an outdoor pen, to simulate the conditions during commercial movement of calves for livestock markets. The transport journey had a duration of 3 h with a space allowance of 1 m^2 per 270 kg and started approximately 2 h after the first sampling. Table 1 summarizes the environmental data present at the time the study was carried out [24].

Table 1. Values for climatic variables during the study period.

Time	AT Min (RH)–Max (RH) [1]
Before loading	3.5 (94.8)–7.3 (81.6)
During transport	7.3 (81.6)–11.6 (67)
After unloading	10.3 (80.2)–11.4 (71.5)
During fasting	6.9 (89.8)–10.3 (80.2)
After 24 h fasting	8 (94.8)–8.3 (92.6)

[1] AT: Air temperature (°C); RH: Relative humidity (%).

2.1. Tympanic Temperature (TT)

TT was used as an indicator of body temperature. Data logger devices (ibuttons, Maxim Integrated Products Inc., San Jose, CA, USA) were manually installed into the tympanic canal using the same procedure described by Arias et al. [25]. Devices were fitted 24 h before initiating the experiment. Data loggers registered TT every 10 min; thus, in order to obtain values for the sample times (before loading, after unloading), the measurements from the 3 h before and after transport were averaged for each calf. The data from two calves and all TT records after the 24 h fasting period were omitted from the analysis as anomalous values were obtained, probably because of device displacement inside the calves' ears.

2.2. Maximum Eye Temperature (MET)

MET was obtained by capturing infrared images of both eyes at approximately 0.3 m distance and a 90° angle from the individual using a thermal camera (FLIR i5, FLIR Systems, Wilsonville, OR, USA), calibrated with an emissivity(ε) of 0.95, according to information provided by the manufacturer. Image analysis was performed using the software FLIR Tools 3.1 (FLIR Systems, Wilsonville, OR, USA) and atmospheric temperature and relative humidity were included in the calculation. The image obtained was used for analysis, as shown in Figure 1. The MET of both eyes was averaged to obtain one MET value for each calf at each sampling.

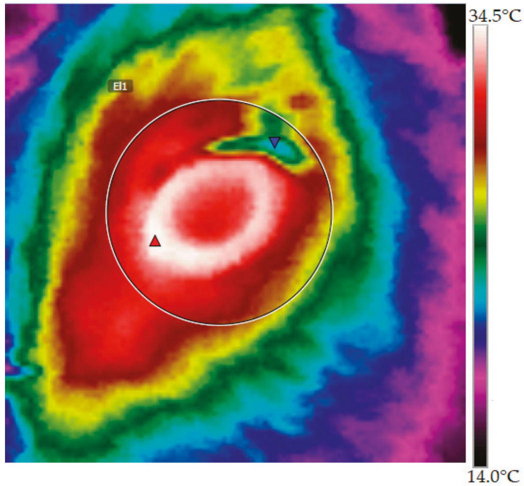

Figure 1. Infrared image of the eye region of a calf. Red triangle indicates maximum eye temperature (MET).

2.3. Blood Variables

Blood samples were collected by jugular venopunction using vacutainer® (needle 20G x1”). Three collection tubes were used: A tube without additives was used for measurements of cortisol and haptoblogin (HPT) serum levels, a tube with EDTA was used for creatine kinase (CK) and betahydroxybutyrate (β-HB) measurements, and a third collection tube with NaF was used for glycemia measurements. All blood samples were placed immediately on ice, then centrifuged (2500 rpm for 5 min) for plasma or serum harvest, and stored at −20 °C for subsequent lab analysis. Plasma cortisol concentrations were determined by chemiluminescent immunoassay radioimmunoassay (CLIA) and glucose plasma concentrations were determined using the GOD-PAP test. The concentrations of β-HB were determined using the enzymatic technique that used the 3-hydroxybutyrate dehydrogenase enzyme, and plasma CK activity was measured using the IFCC and ECCLS kinetic method. Serum haptoglobin concentrations were determined using the commercial kit Tridelta PHASE Haptoglobin Assay (Tridelta Development Ltd., County Kildare, Ireland).

2.4. Live Weight (LW)

All calves were individually weighed after all other measurements were taken, using a mechanical cattle scale (0.5–1000 kg).

2.5. Data Analysis

For descriptive analyses, variables are described by mean and standard error (SE). To assess the variation in the physiological variables measured in calves, after transport and fasting times, linear mixed model (LMM) analyses were performed. The model used was:

$$\gamma ij = \mu + \beta i + \varepsilon ij$$

where γ corresponds to a dependent variable measured; μ is time (three evaluations for each calf), considered as fixed effect; β represents the calf, included as random effect; and ε is the error not explained by the model. Data were analyzed using the lme4 statistical package and multiple comparisons were explored using a Tukey’s adjustment included in the lsmeans function in R software version 3.2.2 [26].

3. Results and Discussion

A significant increase of 0.55 °C in TT was found between the temperatures before loading and after unloading (Table 2). However, a 1.1 °C daily variation can occur even under moderate thermal conditions in beef cattle [27]. The body temperature represents an integrated response to both internal and external factors such as climatic conditions, heat production, and the heat losses that an animal experiences [25]. Moreover, core body temperature can also be dramatically increased with muscular activity, and by nervous and hormonal factors (such as sympathetic nervous activity), catecholamines, and thyroid hormones [28]. The relationship between body temperature and the level of stress, either physical and/or psychological, at several stages of animal handling has also been shown [29,30].

Table 2. Mean (±SE) of all the variables measured before loading, after unloading, and after the 24-h fasting period.

Variable	Before Loading	After Unloading	After Fasting	Reference *
Live weight (kg)	146.1 (±6.0) [a]	141.1 (±5.9) [b]	136.1 (±5.9) [c]	-
Tympanic T° (°C)	37.15 (±0.21) [a]	37.7 (±0.10) [b]	-	-
Maximum eye T° (°C)	34.72 (±0.22) [a]	36.30 (±0.17) [b]	34.48 (±0.19) [a]	-
Cortisol (μg/dL)	1.55 (±0.34) [a]	2.28 (±0.35) [a]	2.29 (±0.36) [a]	0.3–2.0
Glucose (mmol/L)	3.68 (±0.10) [a]	4.38 (±0.14) [b]	3.97 (±0.10) [c]	2.5–4.1
β-HB (mmol/L)	0.26 (±0.02) [a]	0.22 (±0.03) [a]	0.35 (±0.04) [b]	0.1–0.6
Creatine kinase (U/L)	242.9 (±18.9) [a]	398.4 (±46.6) [b]	189.3 (±15.5) [a]	<94
Haptoglobin (mg/mL)	0.16 (±0.01) [a]	0.15 (±0.00) [a]	0.26 (±0.07) [a]	<3 mg/mL

Different letters (a, b, c) represent statistical differences ($p < 0.05$) between sampling times. * Reference values: for cortisol according to DCPAH, Michigan State University [31]; other blood variables according to Wittwer [32].

MET also increased significantly after transport and then returned to initial values after the 24 h fasting period. The rise in temperature after transport was 1.6 °C (Table 2). An increase in MET has previously been reported following castration in calves [33], after jumping competitions in horses [21], and during the veterinary clinic examination phase, compared with both pre- and post-examination phases, in dogs [23]. Nevertheless, some authors described a rapid drop in eye temperature during the minutes following a stressful and/or painful procedure, for example, calves disbudded without local anesthetic [18] and heifers exposed to fear related handling procedures such as being hit with a plastic tube, sudden flag movements, shouting, and being shocked with an electric prod [19]. The rapid drop in eye temperature in cattle during the first seconds after the stimuli has been explained as a sympathetic response, which is part of the autonomic nervous system reaction [17,18]. If the stressor persists for a longer time, the HPA axis produces a cortisol response that could be maintained from minutes to hours. In addition, the nature of the stimulus, or the level of fear and/or pain, that the animals experience may affect the duration of the drop in eye temperature [19]. The results of the present study suggest that transportation was a strong enough stimulus to cause a later increase in eye temperature in calves, as described by Stewart et al. [17,18]. However, no relationship between the increase in eye temperature and HPA axis activation has been shown [33,34]. The increases in MET and TT could reflect a body temperature increase due to stress and physical exercise during transport. It has been proposed that MET, measured with infrared thermography, may be associated with body core temperature [13,35]. In addition, although thermography is described as a non-invasive measurement, in the case of the MET measurements the animals had to be immobilized in a chute, which was undoubtedly a stressful procedure by itself. Otherwise, if there had been a drop in the eye temperature of the calves due to the handling procedures, it would probably have happened during loading, after the current measurement was made. MET in the calves decreased after unloading, returning to initial levels after the fasting period (Table 2). Cattle are a homeothermic species that need to maintain a high metabolic rate to generate heat, and so they require a high level of feed intake [36].

Regarding the blood variables; cortisol and haptoglobin did not change significantly after 3 h of transport or after 24 h of fasting (Table 2). When animals are transported, the effects of this process can be assessed by monitoring glucocorticoid concentrations, but the limitations described for this stress indicator, such as the diurnal fluctuation in plasma cortisol concentration and the effects of the sampling procedure itself, may prevent a correct evaluation [10,37]. However, all mean levels were higher than reference values for cortisol after unloading and after the 24 h fasting period, probably due to HPA axis stimulation during sampling. Animal scientists have hypothesized that glucocorticoids may have an impact on the production of acute phase proteins such as haptoglobin [9]. Literature suggests that haptoglobin is less sensitive and slower reacting than other acute phase proteins, like serum amyloid A (SAA) [38,39]. Werner et al. [8] showed a significant increase in haptoglobin levels 24 h after unloading beef calves transported for a duration of 63 h.

Creatine kinase increased significantly after transport and returned to initial values during fasting in pens (Table 2). CK appears in the circulating plasma because of tissue damage (e.g., bruising), and also when there is vigorous exercise, and is relatively organ specific (i.e., muscle) [37]. In this study, calves were transported at a low stocking density which probably meant that an additional physical effort from calves was required in order to maintain balance during transport. Additionally, this same factor could have influenced bruising when the truck was in motion. The significant decrease in CK after the 24 h fasting period is likely associated with the elimination of the physical stressor, and a rise in excretion, as observed previously in steers after transport for slaughter [40]. CK values were higher than reference values (Table 2) at all sampling times, possibly because of previous handling, such as immobilization for sampling.

After strenuous exercise, ketone oxidation by muscles is reduced and this leads to an increase in plasma levels of betahydroxibutyrate [9]. Additionally, the high weight loss observed was consistent with the significantly higher concentration of β-HB found after the 24 h fasting period (with transport included). This indicated that, even with the short time off feed, the calves had to make use of their body reserves, even though mean β-HB values were within normal ranges for the species for all sample times (Table 2).

Blood glucose levels reflect the nutritional status of an animal, and during food deprivation the glucose blood levels decrease [41]. The results here show that transport may have had a greater impact on plasma glucose than food deprivation (Table 2). There were significant differences in glucose values between sampling times (Table 2). Glycemia increased over the reference values after transport and then returned to basal levels, but remained higher than before loading. The maintenance of glycemia may have been associated with the use of liver glycogen, as practically all glucose available to ruminants comes from the glycogenolysis process [42,43]. Increases in glucose levels after transportation and fasting have been previously reported in calves after 8 h of fasting and 8 h of transport [44]. The explanation for this increase is related to the primary response to stress [9], so glucose levels can be used as an indirect indicator of stress [41].

The calves lost 10 kg live weight (LW). Fifty percent of the total LW loss occurred between sampling before loading and sampling after unloading (within approximately 5 h), whereas the other 50% occurred while the calves were fasting in the pen (Table 2). Generally, weight loss during transport is accelerated compared with deprivation of food and water without transport [9]. The calves lost 6.8% of LW in 24 h, which is consistent with Knowles et al. [9] who described an initial loss of LW in ruminants, predominantly due to loss of gut fill, of approximately 7% during the first 18 to 24 h without food and water. Metabolic changes due to stress are greatest if increased heat production and decreased feed intake occur concomitantly. In this situation, the required calories come from the catabolism of tissues [45]. Body weight loss in animals is probably the most significant economic effect of transport [8].

4. Conclusions

From this preliminary study it can be concluded that even in a controlled replication of the marketing process, where calves were weaned a month before the study, were handled calmly, transported at a low stocking density, and fasted without being mixed or exposed to an unfamiliar environment, there was a significant effect of transport and fasting on the calves, which were measurable through MET, TT, blood indicators such as CK, glucose, β-HB and their live weight. This study was limited to a small number of animals, lacked a control group, and was under simulated commercial conditions. Nevertheless, the findings strongly suggest that more extensive studies of calves undergoing true, commercial marketing are urgently required. Exposure to a real marketing situation, with additional real-life stressors such as handling by untrained staff, weaning just before loading or when they arrive in the market, transport both to and from the market, fasting in pens without protection against adverse weather conditions, stocking at higher densities, and getting mixed with unfamiliar animals would undoubtedly produce greater stress, with repercussions for calf welfare and productivity.

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Effect of Two Transport Options on the Welfare of Two Genetic Lines of Organic Free Range Pullets in Switzerland

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Simple Summary: Animal welfare has been of increasing interest to consumers and producers of animal products in Europe. Issues during transport affect both the wellbeing and the productivity of livestock. This study was conducted to analyze two practice-oriented transport variants of organically mixed-held white and brown pullets. No significant difference could be found between the transport variants. Instead, we discovered clear differences between the two genetic pullet lines.

Abstract: The welfare of two genetic lines of organic layer hen pullets—H&N Super Nick (HNS) and H&N Brown Nick (HNB)—was compared during two commercial transport variants of 15 flocks of mixed-reared birds. Birds were either transported overnight (with a break in travel), or were transported direct to the layer farm (without a break in travel). Samples of feces were collected non-invasively from 25 birds of each genetic line per flock for each transport variant before transportation to evaluate baseline values of glucocorticoid metabolites, and at 0 h, 3 h, 6 h, 10 h, 24 h, 34 h, 48 h, 58 h, and 72 h after the end of transportation, to measure transportation and translocation stress. We assessed the fear toward humans with the touch test before transportation, and we checked the birds' body condition by scoring the plumage condition and the occurrence of injuries. Body weight before and weight loss after transportation were determined, and ambient temperature was measured before, during, and after transportation. Stress investigations showed no significant differences between the transport variants (effect: −0.208; 95% confidence interval (CI): (−0.567; 0.163)). Instead, we discovered differences between the pullet lines (effect: −0.286; 95% CI: (−0.334; 0.238)). Weight loss was different between the transport variants (2.1 percentage points; 95% CI: (−2.6; −1.5)) and between the genetic lines, as HNB lost significantly less weight than HNS (0.5 percentage points; 95% CI: (0.3; 0.7)).

Keywords: animal welfare; transport; pullet; stress parameter; corticosterone metabolite

1. Introduction

The World Organization for Animal Health provides a reference document [1] of international standards for animal health and zoonosis; it grants animals kept under human care the internationally recognized "five freedoms" of welfare described as follows: freedom from hunger, thirst and malnutrition; freedom from fear and distress; freedom from physical and thermal discomfort; freedom from pain, injury and disease; freedom to express normal behavior patterns.

The Swiss consumer assumes that organic livestock farming applies more species-appropriate animal husbandry corresponding to the animals' natural living conditions, and thus provides better animal welfare than conventional farming practices [2]. Animal transport is an exceptional situation, because animal welfare may be compromised for a certain time. The European Commission Regulation (EC) No 1/2005 [3], limits the transport duration of poultry to less than 12 h. The Swiss Animal Welfare Regulation has limited the maximum duration of animal transport to less than 8 h since its amendment to Art. 152a (1) of 28 October 2015 [4], like the German Animal Welfare Transport Regulation [5]. Whereas some of the German organic labels further limit the maximum transport durations to less than 4 h or 2 h [6–8], none provide specific requirements for poultry, pullets, or laying hens. Similarly, the Swiss organic labels Bio Suisse [9] and KAG (Consumer-Working-Group; German designation: Konsumenten-Arbeits-Gruppe) [10] refer to the transportation guidelines of the Swiss Animal Protection [11], which are designed for big and small animals, not including economic fowl. All regulations, including EC No 1/2005, disregard loading and unloading time (Table 1).

Various stressors such as climate, environment, nutrition, physical, and social and physiological conditions are likely to influence welfare and performance [12]. The present study examined the effects of transportation and translocation on the stress hormone levels of 18-week-old organically reared pullets of two mixed-held layer lines—H&N Super Nick and H&N Brown Nick—in transit from the rearing farm to the farm of laying hens (novel environment) in Switzerland. The geographically dispersed locations of rearing and laying farms require that hens are transported on the road over distances of varying lengths. We compared two practice-oriented transport variants. Although the transportation process is supposed to be the most stressful environmental challenge experienced by broilers [13], catching and handling of the birds may already have profound effects on the degree of physiological stress, and it may reduce welfare during the upcoming transit [14–16]. Thus, we took into account the entire duration of the hens being held in plastic crates, including loading and unloading time.

To evaluate the stress response to the two transport variants, we measured corticosterone metabolites non-invasively in excretions of the pullets. Fecal corticosterone metabolites have previously been used as a reliable indicator of adrenocortical activity [17].

The project was initiated by Switzerland's largest organic egg supplier—hosberg AG in Rüti—and was supported by two other Swiss-based companies—Wüthrich Brüterei AG in Belp and Prodavi AG in Schötz. Participation in "LTK (Institute of Laboratory Animal Sciences) Module 2: Training for Persons Responsible for Directing Animal Experiments" was required as a prerequisite for the intercantonal approval by the Swiss Cantonal Veterinary Office in Zurich, and the project was approved on 6 January 2016, under registration number ZH196/15. The study refers to Europe, with special emphasis on Switzerland.

Table 1. Comparison between Switzerland, Germany and Austria regarding maximum stocking densities of pullets and laying hens per pen, as well as minimum space requirements and maximum duration in the transit of pullets according to transnational (European Union, EU), federal, and label-specific regulations. STS = Swiss Animal Protection ("Schweizer Tierschutz"). All numbers in square brackets are references.

Regulation	Country level							
	Switzerland			Germany		Austria		EU
	Federal	Label: Bio Suisse	Label: KAGfreiland	Federal	Label: Demeter, Bioland, Naturland	Federal	Label: Bio Austria	
Maximum Stocking Density Per Pen								
Pullets	No specification [4]	4000 [9]	4000 [10]	No specification [5]	3150 [6]	4800 [18]	4800 [19]	4800 [20]
Layers	18,000 [4]	2000 [9]	2000 [10]	6000 [5]	3000 [6–8]	3000 [18]	3000 [19]	3000 [20]
Transport								
Minimum Space in Transport Cage	160 cm^2/kg for <3.0 kg [4]	According to Transport Guideline of STS [9]	According to Transport Guideline of STS [10]	200 cm^2/kg for <1.0 kg 190 cm^2/kg for <1.3 kg 180 cm^2/kg for <1.6 kg 170 cm^2/kg for <2.0 kg [5]	No specification [6–8]	180–200 cm^2/kg for <1.6 kg 160 cm^2/kg for <3.0 kg [18]	180–200 cm^2/kg for <1.6 kg 160 cm^2/kg for <3.0 kg [19]	180–200 cm^2/kg for <1.6 kg 160 cm^2/kg for <3.0 kg [20]
Maximum Transport Duration	8 h [4]	According to Transport Guideline of STS [9]	No specification for pullets [10]	No specification for pullets [5]	4 h [6–8]	No specification for pullets [18]	6 h [19]	12 h [3]

2. Animals, Materials and Methods

2.1. Experimental Design

The study was conducted with organically mixed-held pullets of two genetic lines—H&N Super Nick (HNS) and H&N Brown Nick (HNB)—of a commercial breeder and distributor (H&N International, Cuxhaven, Germany). Parent animals were imported to Switzerland and raised as organic laying hens. The experimental unit consisted of pullets and laying hens of 15 flocks, which were reared and kept according to the guidelines of Bio Suisse (Association of Swiss Organic Farming Organizations, Basel, Switzerland) [9] on free-range farms. Each rearing farm raised 4000 pullets, and farms of laying hens kept 2000 birds. The average ratio between HNS and HNB normally was 50:50 to 60:40. The transport to the farm of laying hens was realized at the age of 18 weeks. The study was based on two practically relevant commercial transport variants—with and without transportation break—which were categorized according to distance and length. Variant I "transport overnight" (transportation was performed with break) was compared with Variant II "direct transport" (transportation was performed without break). On average, 2014 birds were transferred on each transit. Each plastic crate (90.5 × 61.5 × 31.5 cm) was loaded with 16 pullets according to the Swiss Order on the Protection of Animals [4]. Because the start of loading also means the starting point of stress, we defined the time from the beginning of loading until the end of unloading as "time in plastic crate" or transport duration. Thus, the average transport duration was 13.5 h for Variant I and 5.0 h for Variant II, whereas the mean journey time alone was 2.6 h for Variant I and 1.0 h for Variant II (time on the road). Loading regularly began at 7 p.m. The legally prescribed transport duration was never exceeded [4]. We timed our investigation to include winter, spring, and summer.

Temperature was measured with HOBO U10 (temperature data loggers, Onset Computer Corporation, Bourne, MA, USA) inside of the stable at animal head height and during transportation inside of the plastic crates on the upper edge. For both transport variants, temperature was recorded during the whole investigation period every 10 min per flock. Means of minimum and maximum temperature values during the testing period (January until July) for Variant I ranged from 11.1 to 32.3 °C (rearing farm), 1.9 to 34.7 °C (transportation vehicle) and 3.8 to 34.1 °C (farm of laying hens) and for Variant II from 7.6 to 26.3 °C (rearing farm), −8.9 to 28.8 °C (transportation vehicle), and 6.1 to 26.2 °C (farm of laying hens).

Animal husbandry varied according to the individual farmer's management.

2.2. Corticosterone Monitoring

To examine the effects of transportation and translocation on stress in each flock, corticosterone levels were measured non-invasively by extracting metabolites from bird droppings. For each sampling time point, 25 pullets of each genetic line were randomly caught from different tiers of the dimmed barn. To enable the collection of individual, spontaneously voided droppings, the pullets were placed separately in cleaned and disinfected plastic crates, and marked on their legs with a pen (Edding-Egg-Color-Pen, Wunstorf, Germany). Samples were collected within 1 h of the experimenter entering the barn according to Rettenbacher et al. [21], who found a first major peak 1 h after a stress pulse in laying hens. One dropping per bird was collected immediately after defecation, put into frost-resistant plastic bags and frozen on dry ice at a usual temperature of −78.5 °C. Droppings were transferred to a freezer after sampling. To determine baseline concentrations, pullets were sampled at 9:00 a.m., two days before transportation. For measurements of transportation and translocation stress, further droppings in both variants were collected 0 h, 3 h, 6 h, 10 h, 24 h, 48 h and 72 h after transportation. Initially, the flocks had been sampled 9 h and 12 h (instead of 10 h) after transportation. However, at these time points, only a few (if any) birds defecated. To prevent an unworkable additional work load and a possible violation of the numerical limit of permitted experimental birds, we decided to collect samples 10 h after transportation. Taking the circadian rhythm into account, flocks of Variant

II were sampled additionally 34 h and 58 h after transportation. Altogether, 5751 droppings were collected and analyzed.

In the laboratory, 0.5 g of each sample was suspended in 5 mL of 60% (*v*/*v*) methanol by shaking for 30 min on a multi-vortex (RapidVap, Labconco, Kansas City, MO, USA) [22]. When a smaller portion had to be used, an aliquot of methanol was added. After centrifugation (GS-6KR Centrifuge, Beckman, Krefeld, Germany) for 15 min, aliquots of the supernatant were diluted 1:10 with assay buffer, and concentrations of fecal corticosterone metabolites (CM) were determined with a cortisone enzyme immunoassay [21]. The applied method has been validated physiologically and biologically for chickens by Rettenbacher et al. [21,23].

2.3. Hen-Human Relationship: Touch Test

The level of fear of humans is an important determinant of welfare of pullets and laying hens. Regular handling of pullets is fear reducing [24], and positive additional human contact of laying hens reduces their fear level and influences their corticosterone level in blood positively [25]. In contrast, fear-inducing humans reduce the wellbeing of animals [26]. Accordingly, we tested each flock on avoidance and approach behavior by using the touch test of Raubek et al. [27] to assess the birds' reaction to an unfamiliar human. The test was performed with each flock by the same test person, who was unfamiliar to the flock before test. The test person wore protective clothing such as a blue overall, plastic overshoes and a hair cloth. Tests were carried out in the roofed outdoor run area (winter garden) of the rearing farm. Entering the winter garden was the initial contact between flock and test person. The unfamiliar test person moved slowly—one step per second—through the winter garden, approached a group of at least three pullets, squatted for 10 s and then counted all pullets within one arm's length around her. Thereafter, the test person tried to touch one bird after the other. The test was carried out until 33 groups were examined. Any attempt to approach a group or squat down was counted, even if all pullets retreated from the test person [28].

2.4. Body Condition

The body condition of the birds was evaluated by scoring the plumage and integuments before and after transportation, following feces sampling and the touch test. The assessment basis was the LayWel grading scheme [29] modified according to Schwarzer et al. [30] for pullets. Plumage condition was divided into four degrees of severity (4 = no damage, 3 = 1–5 damaged feathers, 2 = >5 damaged feathers, 1 = plucked area >1 cm). A higher score equaled a better plumage condition. This was assessed on seven individually scored body areas, resulting in a maximum pooled score of 28. Damage of flight feathers, tail feathers, and the presence of fault bars were evaluated separately with binary scores (0 = negative and 1 = positive). Originating from a total plumage score of 28, a bad feather cover was indicated by ≤11–14, and a good feather condition by ≥18–20. Injuries were divided into three degrees of severity (0 = negative, 1 = Ø ≤0.5 cm, 2 = Ø >0.5 cm) on 10 individually scored body areas. Injuries of the comb, head and eyelid were evaluated separately with binary scores (0 = negative and 1 = positive).

2.5. Live Weight

To check minimum body weight, which is 1300 g for HNS and 1479 g for HNB at the age of 18 weeks (according to the breeder and distributor H&N International), 50 numerically marked birds, 25 per line, were weighed during loading. To check a possible transport-related weight reduction due to water and feed withdrawal and "time in plastic crates," we compared body weights before and after transportation (following sampling) for each hen. Results were compared with a control flock that was not transported and was kept overnight in the winter garden without access to water and feed but were free to move around; hens of the control flock were weighed in the evening (8:00 p.m.) and in the morning (7:00 a.m.). For evaluation, the weight of the same numerically marked bird was compared in each case. Weight determination was carried out with a BAT1 poultry scale (VEIT Electronics, Moravany, Czech Republic). The 50 hens of the transport study were divided into four transport crates. To ensure a regular number of 16 birds per crate according to the Swiss Order on the Protection of Animals [4], the transport crates were supplemented with non-weighed birds.

2.6. Statistical Analysis

For the statistical analysis, the relationships between the predictors (transport variant, layer line, flock) and the response variables (baseline CM concentration, CM concentration after transport, returned to baseline value after 72 h, and difference in plumage score before and after transport, transport weight) were analyzed simultaneously using mixed-effects models. The flock was modeled as an unstructured random effect for the model constant (intercept), and the transport variant and the layer line were modeled as ordinary fixed effects. For the continuous response baseline CM concentration, CM concentration after transport, difference in plumage score before and after transport, and transport weight, normal distributions were chosen as observation models. For the binary outcome return to baseline value after 72 h, a logistic regression model was used. Results from this analysis were expressed as odds ratios (OR). For baseline CM concentration, temporal progression was also considered by including time as an unstructured random effect (in contrast to a temporal effect, because of very few unequally distributed time points).

Data were analyzed by using the statistical programming language R [31]. All (generalized) mixed-effects models were estimated by the integrated nested Laplace approximation approach [32] within a fully Bayesian setup.

3. Results

3.1. Corticosterone Monitoring

Mean baseline concentrations of excreted CM in the examined flocks were 43 ng/g and 66 ng/g for Variant I and Variant II, respectively. Overall, birds of Variant II had higher baseline values than birds of Variant I (Table 2). However, differences were not significant (effect: −18.6; 95% confidence interval (CI): (−45.3; 9.1)).

Table 2. Comparison of baseline levels (mean ± SEM) of corticosterone metabolites measured in transport Variants I and II in H&N Super Nick (HNS) and H&N Brown Nick (HNB) animals, and in total for both layer lines.

Variant	Line	Baseline Value (ng/g)	SEM (ng/g)	Baseline Value Total (ng/g)	SEM (ng/g)
I	HNS	57	3	43	2
	HNB	30	2		
II	HNS	93	8	66	4
	HNB	40	3		

HNB individuals had significantly lower baseline values than HNS birds (effect: −37.8; 95% CI: (−45.4; −30.3)). Three flocks showed significant deviations from mean baseline values in Variant II: two flocks with significant above-average values (D2 and D7) and one with significantly lower values (D6). Any effect that crossed zero did not significantly deviate from the mean baseline value in this flock (Figure 1).

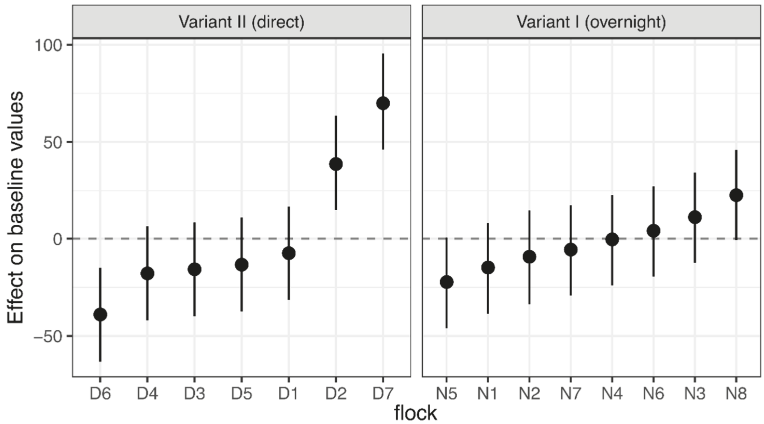

Figure 1. Linear mixed-effects model. Effect of the flock on the baseline concentrations of corticosterone metabolites in either two transport variants, modeled with the flock as an unstructured random effect for the model constant, and with the transport variant and the layer line as fixed effects. The figure shows estimated values (circles) with their 95% confidence intervals (bars) of the effect of flock on baseline concentrations. Estimates that do not cross zero deviate significantly from the baseline concentration.

The transit from the rearing farm to the farm of the laying hens resulted in higher mean concentrations of CM compared with the mean baseline values. The highest values were found immediately on arrival (0 h). The mean concentration at 0 h was 173 ng/g and 323 ng/g for Variant I and Variant II, respectively. CM concentrations decreased rapidly during the 0–6 h interval after transportation. Variant I showed an increase during the 6–12 h interval, followed by a steady decline. Values for Variant II were slightly increased at 24 h, 48 h, and 72 h, and slightly decreased at 34 h and 58 h, with the additional sampling times considering the circadian rhythm (Table 3).

Table 3. Minimum (Min), maximum (Max), and mean concentrations ± SEM of corticosterone metabolites (CM) after transportation in two transport variants.

Variant	Hours after Transportation	Min CM (ng/g)	Max CM (ng/g)	Mean CM (ng/g)	SEM (ng/g)
I	0	4	1337	173	22
	3	4	570	111	7
	6	3	440	61	7
	9	5	568	96	5
	10	4	459	69	6
	12	11	315	128	3
	24	4	786	92	4
	48	3	402	73	3
	72	2	245	64	3
II	0	4	2215	323	11
	3	4	967	134	5
	6	3	992	112	3
	10	4	590	89	11
	24	5	632	95	4
	34	4	307	67	9
	48	5	462	86	5
	58	4	330	69	3
	72	4	456	74	2

We found no significant difference in CM concentrations between Variant I and Variant II, because the overall trend was similar, and confidence intervals overlapped strongly (effect: −0.208; 95% CI: (−0.567; 0.163)) (Figure 2).

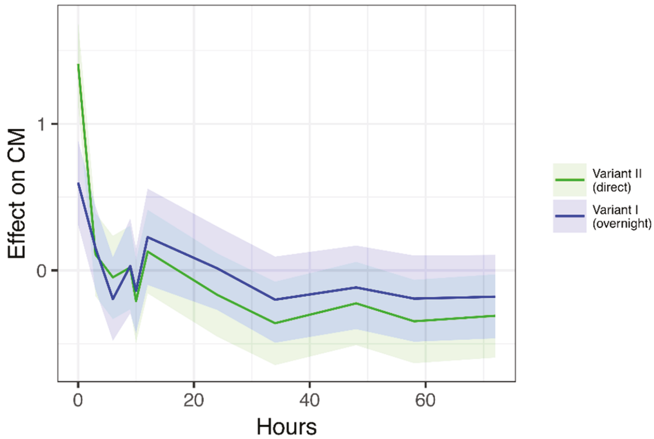

Figure 2. Linear mixed-effects model. Effect of time after transportation (hours) on concentrations of corticosterone metabolites (CM), considering the flock as a random effect, and the transport variant and the layer line as fixed effects. Estimated temporal progression of CM concentrations, shown as estimated effects (solid lines) and 95% confidence intervals (shaded areas) for each transport variant.

Significant differences in CM concentrations were found once again between the layer lines. HNS had higher values than HNB (effect: −0.286; 95% CI: (−0.334; −0.238)). Furthermore, considerable variations among flocks were found. Significant above-average values could be measured for one flock of Variant II (D7), significantly lower values for three flocks of Variant II (D5, D3 and D4), and one flock of Variant I (N4) during the 0–72 h interval. Any effect that crosses zero does not significantly deviate from the mean CM concentration in this flock (Figure 3).

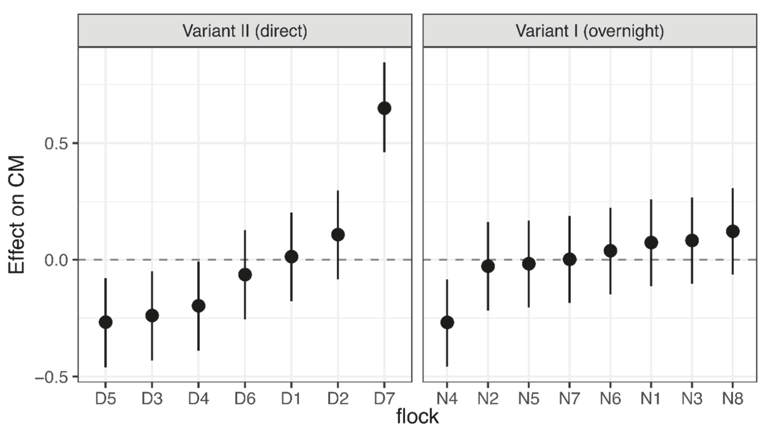

Figure 3. Linear mixed-effects model. Effect of the flock on concentrations of corticosterone metabolites (CM) 0–72 h after transportation in either two transport variants, modeled with the flock as an unstructured random effect for the model constant, and with the transport variant and the layer line as fixed effects. The figure shows estimated values (circles) with their 95% confidence intervals (bars) of the effect of flock on CM concentrations. Estimates that do not cross zero deviate significantly from the mean value.

The ratio of CM concentrations after 72 h to CM baseline values showed that most CM concentrations did not return to baseline values in both transport variants (Figure 4). For both layer lines, we could not find a significant effect of transport variant on the return to baseline values (effect: 0.43; 95% CI: (−2.41; 3.26) and −1.49; 95% CI: (−4.34; 1.37) for HNS and HNB, respectively).

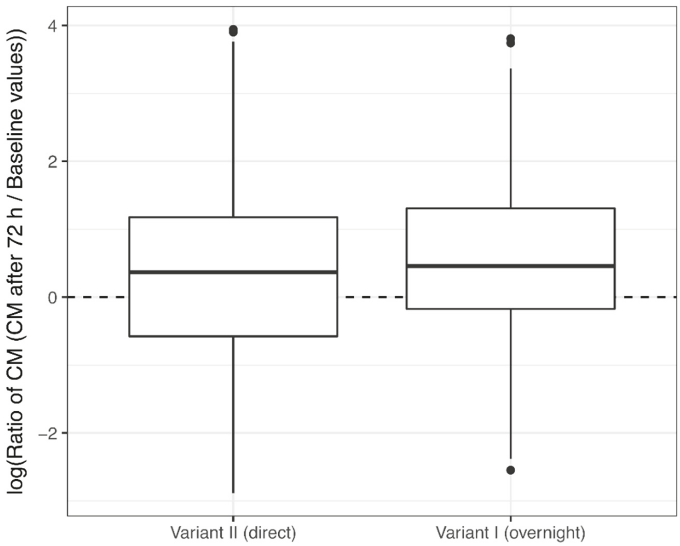

Figure 4. Ratio of concentrations of corticosterone metabolites (CM) after 72 h to CM baseline concentrations in two transport variants. Thick black lines show mean ratios, boxes represent upper and lower quartiles, whiskers represent 95% confidence intervals, and dots show outliers. A mean ratio above zero indicates an increase in CM concentration relative to the baseline value, whereas a mean ratio below zero indicates a decrease.

3.2. Hen-Human Relationship: Touch Test

With the touch test, we evaluated the hen–human relationship based on the approach and avoidance behavior of the birds between flocks. To evaluate whether this behavior was reflected in the measured CM concentrations, we compared CM concentrations between hens that stayed an arm length away from the examiner, and those that could be touched. An increase in CM concentration by one unit (1.0 ng/g) resulted in a significantly greater number of hens that could be touched (effect: 0.004; 95% CI: (0.001; 0.006)). In addition, a few differences in approach and avoidance behavior between flocks were found: One flock (D5) had significantly fewer hens that could be touched compared with three other flocks (D2, D6, and N2).

3.3. Body Condition

The examined flocks of both transport variants showed an average plumage score of 24.62 ± 1.37 (mean ± SD) before and after transportation, indicating a good feather condition (maximum possible score = 28, for seven body areas with four degrees of severity). Flocks D4 and D5 of Variant II had a better plumage score after transportation than before (Figure 5).

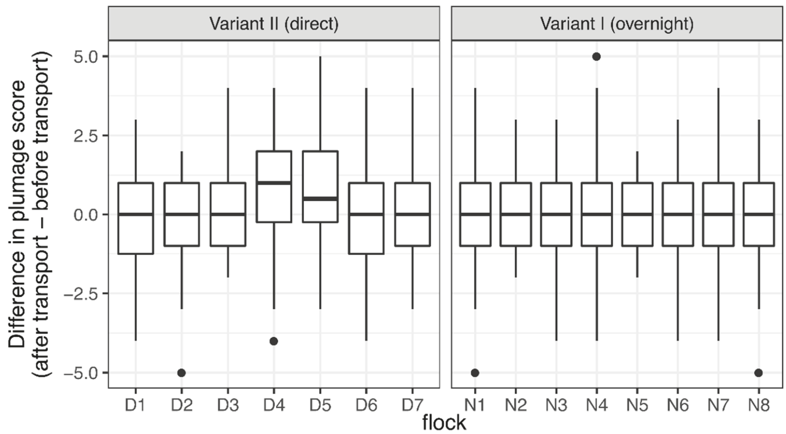

Figure 5. Difference across flocks in plumage score before and after transportation in two transport variants. Thick black lines show mean values, boxes represent upper and lower quartiles, whiskers represent 95% confidence intervals, and dots show outliners. A mean value above zero indicates a better plumage score after than before transportation.

Altogether, we found no significant differences in the plumage condition of the body areas scored with four degrees of severity before and after transportation, regardless of layer line, transport variant or flock, with one exception: HNB in comparison with HNS showed less plumage deterioration (−0.28, 95% CI: (−0.8; 0.25)) in Variant I, and in Variant II, greater deterioration (0.35, 95% CI: (−0.03; 0.73)). The total plumage score, including those body areas scored with two forms of severity (flight and tail feathers, fault bars) apparently significantly improved after transportation compared with before transportation in Variant II (OR: 0.672; 95% CI: (0.53; 0.863)) but not in Variant I (OR: 1.454; 95% CI: (0.931; 2.218)). Integument injuries of body areas scored with three degrees of severity were not sufficiently variable in their distribution of characteristics, and only isolated injuries were found. The same applies for integument injuries of the eyelid (binary score). Both comb and head (evaluated with a binary score) were scored positive in 13% and 4% of the cases, respectively. We could find no major differences in integument injuries before and after transportation.

Following the transit from the rearing farm to the farm of laying hens, the birds showed a weight loss of −2.9% ± 1.9% (mean ± SD). Comparing both transport variants, birds of Variant I lost significantly more weight (2.1 percentage points; 95% CI: (−2.6; −1.5)) than birds of Variant II. Regarding the layer lines, HNB lost significantly less weight than HNS (0.5 percentage points; 95% CI: (0.3; 0.7)). Considering the transport variants, differences in weight loss between layer lines can solely be found for Variant II: HNS showed higher loss in weight (−2.38% ± 1.46%) compared with HNB (−1.3% ± 0.71%) (effect: −0.01603; 95% CI: (−0.02026; −0.01180)). Differences in relative weight losses between flocks hardly existed (Figure 6). None of the mean temperature variables on the rearing farm, the transport vehicle, and the farm of laying hens showed a significant effect on the change in body weight.

Birds of the control flock, which were not transported and were kept in the winter garden overnight, free to move around without access to food and water, showed a mean weight loss of −5.9% (95% CI: (−6.3; −5.6)). HNB hens of the control flocks lost significantly less weight (−5.4%; 95% CI: (−5.8; −5.0)) than HNS hens (−6.5%; 95% CI: (−6.9; −6.1)). Comparing the weights of all birds (study and control flocks) calculated as means, birds of the control flock showed on average a higher loss of −2.0% (95% CI: (−2.5; −1.6)) than birds of the study flocks.

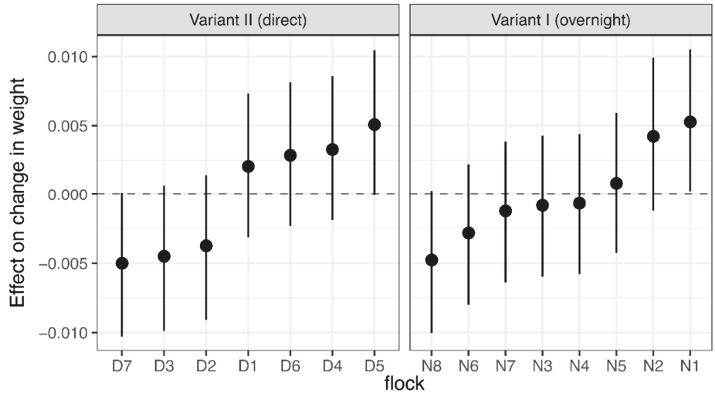

Figure 6. Linear assorted regression model. Estimated body weight differences across flocks before and after transportation in two transport variants, considering the factors of the layer line, the transport variant, and the average temperature on the rearing farms. The figure shows estimates (solid circles) and 95% confidence intervals (bars) of the effect of flocks on body weight changes. Estimates that cross zero do not deviate significantly from the mean values.

The target weight of the 18-week-old pullets, which is defined by the breeder and distributor H&N International, is set at 1300 g for HNS and 1479 g for HNB. It was not reached by all weighed hens: HNS hens weighed on average 1339 ± 102 g (mean ± SD), and HNB hens 1679 ± 156 g. With age included, no differences could be found within each layer line in reaching the target weight (Figure 7). The only significant effect was the effect of the layer line. The chance of HNS observing a shortfall was on average elevated by a factor of 8.1 (95% CI: (5.1; 12.7)).

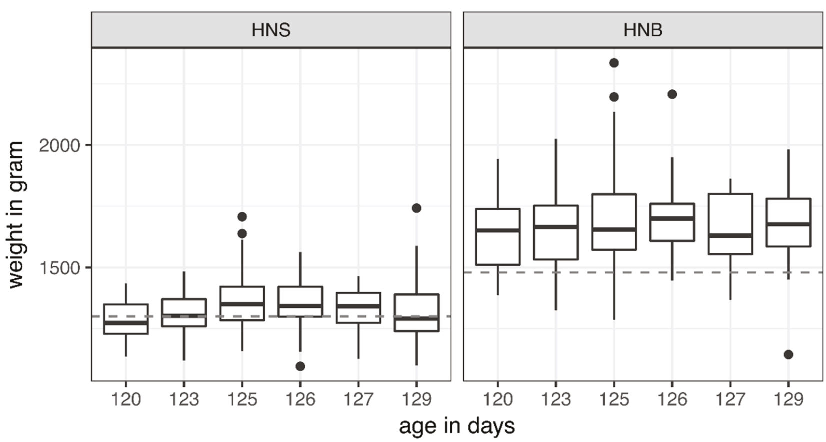

Figure 7. Body weight before transportation in two layer lines, according to age in days. Thick black lines show mean values, boxes represent upper and lower quartiles, whiskers represent 95% confidence intervals, and dots show outliers. The dashed lines represent the target weight according to H&N International for layer lines H&N Super Nick (HNS; 1300 g) and H&N Brown Nick (HNB; 1479 g).

4. Discussion

To the best of our knowledge, this is the first study comparing two practice-oriented transport variants for pullets over a period of 72 h via fecal CM. The aim of the study was to evaluate which of the two examined transport variants resulted in less pronounced stress responses of the birds.

Measurements of CM, a reliable indicator of stress [17], showed no significant transport-specific difference between Variants I and II. Instead, we discovered significant differences between the layer lines in CM responses, touch test results, and weight loss. Several studies have already shown differences between white and brown hens; for example, differences in plasma corticosterone responses after a treatment [33], in tonic immobility [34–37], or in results from other fear tests [38,39].

4.1. Corticosterone Monitoring

Baseline values of CM concentrations and values measured between 0 h and 72 h after transportation did not show significant differences between the two transport variants. Instead, we found significant differences between the two layer lines in both baseline values and values measured after transportation. However, other studies found similar baseline plasma corticosterone concentrations in brown and white layer lines [33,40]. One study analyzed translocation stress in ISA Brown (name of hybride) hens for 36 h after a 1 h long transportation, and found the highest plasma corticosterone concentrations 4 h after transportation [23]. In contrast to this finding, the HNS and HNB hens of our study showed a rapid decrease in CM concentration during the first 6 h after transportation, but just a few returned to baseline values at the end of the study, which might be due to the novel environment. HNS had higher CM levels than HNB at all times in almost every flock. Fraisse and Cockrem [33] reported similar results after 15 min of repeated handling. White hens of their study also showed higher corticosterone levels than brown hens, but only for plasma corticosterone, whereas fecal CM concentrations did not differ between layer lines. At 9 h and 12 h after transportation, CM concentrations in Variant I of our study showed an increase from 61 ng/g at 6 h after transportation, to 96 ng/g and 127 ng/g, respectively, with an intermittent decrease at 10 h (69 ng/g), followed by a steady decline (Table 3). Samples at 9 h and 12 h were taken solely from Flocks N1 and N2; for Flocks N3 to N8, we reduced the sample collection to once at 10 h after transportation. During the investigation period of 72 h, CM concentrations never fell below the value measured 6 h after transportation. In Variant II, we found slight fluctuations of CM concentrations at 34 h and 58 h (additionally taken samples), indicating natural variation due to the circadian rhythm during a 24 h interval. De Jong et al. [41] found a plasma corticosterone peak at 4 h of the 8 h light period during a 24 h investigation on 5-week-old broilers that were fed ad libitum and showed low plasma corticosterone levels during the dark period for 12 h. This finding is contrary to the results from Variant II of our study because samples at 24 h, 48 h and 72 h were taken during the dark period (between 11:00 p.m. and midnight) and samples at 34 h and 58 h were taken during the light period (9–10:00 a.m.). Differences between individual flocks might be attributed to the so-called "passage effect": Management and processes of transportation, for example, differed. Further investigations are necessary to better understand these differences.

4.2. Hen-Human Relationship: Touch Test

The relationship between animals and humans is an important aspect of animal welfare. Additional contact to humans can positively influence the hen–human relationship [28]. Studies on laying hens showed that additional positive contact with a person resulted in reduced fear toward this person [25,28,42] and in a decrease of plasma corticosterone levels [25]. The pullets of our study behaved contrarily to these findings: Pullets with increased CM concentrations were more likely to allow touch by the test person than pullets with low CM concentrations. Four flocks deviated from the average test results. However, these flocks did not show deviations in any of the other study parts. We therefore cannot relate the tameness of these flocks to other test results of this study.

4.3. Body Condition

The examined flocks were in good condition before and after transportation, as measured by the use of the sum of the body parts that were individually scored for plumage condition and integument injuries [43]. With regard to the temporal effect (before and after transportation), we noted a slight improvement to both the plumage and integuments. The main reason is likely to be an insufficient sample size, to ensure that representative estimates and observer deviations are conceivable.

Birds lose weight overnight, even without transportation, and this has potential side effects such as increased corticosterone levels or heat stress, as results from our control flock show. Birds of the control flock were able to move around in the winter garden without access to food and water, matching the lack of these resources for transported birds. "Time in winter garden" for the control flock was 11 h, and this is thus based on the "time in plastic crates" of Variant I. Several studies describe a diurnal and seasonal weight fluctuation in wild birds (e.g., [44–47]) with amplitudes of 5–15% [44,45,47]. The weight loss of the hens of our control flock (−5.9%) falls within this range. However, a mean loss of −3.9% ± 1.8% overnight, as measured for the transported hens of Variant I, is lower than the loss measured in wild birds. Amplitudes in winter (long and colder nights) are higher than in summer [47]. The mean temperature during transport of the studied flocks was 16 °C, whereas the mean outside temperature for the control flock was 19.5 °C. Unfortunately, none of our other study experiments were performed on the control flock. Explanations therefore remain speculative. Scholtyssek et al. [48] found an greater loss of weight in broilers with increasing durations of transportation (1.3%, 2.3%, and 3.1% after transit durations of 1.5 h, 3.0 h and 4.5 h, respectively) whereas another study did not find weight differences between the control and 4 h transported treatment groups [40].

5. Conclusions

Our findings prove that no significant differences exist between the two studied transport variants. This conclusion may be supported by further investigations. Considering the tested flocks, we can say that both transport variants exerted a similar level of stress on the birds. Significant differences between the two layer lines indicated that HNS hens would benefit from transportation in the short variant, whereas stress levels in HNB hens were similar in both variants. Nonetheless, we cannot say whether a longer time of transportation exerts more and longer lasting negative impacts than a shorter period of transportation. Future studies comparing weight development or egg production and egg weight between both transport variants could help to answer the remaining questions.

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animals

Article

Thermal Micro-Environment during Poultry Transportation in South Central United States

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Simple Summary: This project monitored the internal micro-environments of live poultry transport trailers during loading and transport. For the 28 trips evaluated, trailers were modified using common USA industry mitigation practices designed to optimize bird comfort under a wide range of environmental conditions. In the cold season, double boarding of the exterior area of the transport modules elevated the internal temperature more than 8 °C above ambient temperatures as low as −16 °C. However, the temperature elevation may not be sufficient when ambient temperature was below 0 °C. In the warm season, surface wetting of birds and evaporative cooling applied during on-farm loading maintained trailer thermal conditions at or below ambient temperature for part of the road transport. However, this study suggests that additional improvement in equipment design or management is warranted when temperatures are extremely cold or hot.

Abstract: This observational study was conducted to characterize the thermal micro- climate that broilers experienced in commercial poultry transporters under various weather conditions and typical management practices in the South Central USA. We continuously monitored temperature and relative humidity in 45 interior locations of 28 fully-loaded commercial trailers over 2 year spans from 2015–2016 in South Central USA. In the cold season, double boarding of the exterior area of the transport modules maintained temperatures at least 8 °C warmer than ambient temperatures as low as −16 °C. Overall, temperature at all locations decreased as transporters traveled from farms to processing plants during winter trips with double boards. In the hot season, assistance by evaporative cooling during on-farm loading resulted in interior temperatures within ± 2 °C of ambient conditions (up to 36 °C) during road transport. In the summer months, trailers uniformly gained 2 °C as vehicles travelled for 45 min from farms to plants. Apparent equivalent temperatures of the monitored summer trips averaged 80.5 °C, indicating possible heat stress conditions based on the thermal comfort zones defined by literature index values. For longer trips, cooling assistance on the farms may be insufficient to prevent temperatures from rising further into extremely hot conditions in the transporters, leading to a dangerous thermal environment.

Keywords: broiler transport; thermal micro-environment; heat stress; animal welfare

1. Introduction

Market broilers face a variety of stresses, including feed and water withdrawal, vehicle vibration, and noise during live hauls from farms to processing plants. Among these, complex thermal environments have been identified as a major factor inducing physiological stress [1–9], with the most stressful stimuli being the extremes of heat and cold, contributing to seasonal-elevated "dead on arrivals" (DOAs). The welfare of the birds may be compromised either by a combination of air movement, low ambient temperatures (T) and winter precipitation that may cause cold stress, or the more prevalent problem of heat stress during transport [3].

Previous studies have attempted to characterize the behavioral and physiological responses of poultry in transportation in either field or lab conditions [1,6–8,10]. In a Canadian study, the transportation of three to four hours at temperatures below 0 °C was severe enough to decrease the internal body temperatures in broiler chickens [11,12]. Burlinguette et al. [8] reported a near-uniform temperature profile for trailers when ambient temperature was in the range of 8 to 11 °C operated in Saskatoon, Canada. However, when roll-up-style tarpaulin side curtains and most roof vents were closed at ambient temperature below −20 °C, temperature variation as large as 40 °C was observed at different modules in trailers. Ritz et al. [13] recorded black globe temperatures on 24 summer transport trailers in southeast United States, and reported that transport did not appear to exacerbate the temperatures experienced by broilers when the trailers were kept moving.

Ideally, the exposure temperature of broilers should be within the thermoneutral zone of the birds, i.e., within the range of the conditions under which a bird can control its body temperature without altering its metabolic rate. Webster et al. [5] suggested a thermoneutral range for a well-feathered broiler from 8 to 18 °C under higher density in moving vehicles, lower than that of the birds in a typical rearing environment (24 to 28.5 °C) [14].

Quantifying heat loss of broilers on live-haul transport trailers is important to the understanding of the welfare of the broilers during this process [15], but is difficult to accomplish [16]. As an alternative, thermal comfort indices have been developed to assess the impact of the thermal environment on thermoregulatory status of animals. Mitchell et al. [4] developed the apparent equivalent temperature (AET) by incorporating the effect of temperature and humidity as an integrated index to correlate to changes of core body temperature under various T and humidity combinations (Figure 1).

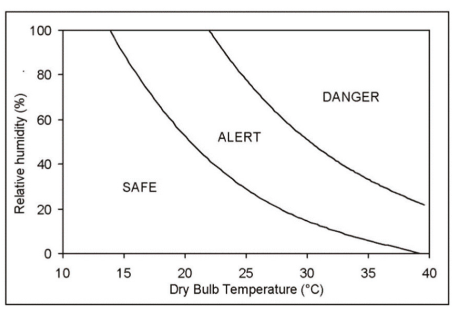


Figure 1. Thermal Comfort Zones for broiler transport defined by apparent equivalent temperature (AET) by Mitchell et al. [4]. Safe limit AET < 40 °C; danger limit Apparent equivalent temperature (AET) > 65 °C. Alert zone indicates moderate thermal stress with some degree of hyperthermia and acid-base disturbances; danger zone indicates severe thermal stress.

The commercial vehicles used to transport broilers in South Central USA differ in their design from those used in Europe or Canada in terms of module materials, size, and weatherization measures. Trip lengths, bird market weight and seasonal stocking density may be different as well [5,8,10]. The objectives of the current study were to characterize the thermal micro-environments encountered by broilers in commercial poultry transporters under various weather conditions and typical management practices in the South Central USA.

2. Materials and Methods

2.1. Trailer Description

This study was carried out in Northwest Arkansas in collaboration with commercial companies during 2015 and 2016. In Southern USA, broilers are transported by galvanized steel modules (2.4 × 1.17 × 1.2 m) arranged on a flatbed trailer measuring 14.4 × 2.4 m (Table 1). Twenty-two modules are stacked in 11 rows on the trailer (Figure 2), with modules stacked in two tiers. Each module consists of a sheet metal roof and 10 perforated compartments arranged as five by two drawers (referred to 10-door cages). Each compartment had a solid plastic floor and a hinged solid door at the front of the compartment (1.2 × 0.21 m) for loading and unloading chickens.

Table 1. Dimensions (m) of the commercial broiler transporters, modules and drawers used in this study.

Component	Length	Width	Height
Trailer	14.4	2.4	2.5 [1]
Module	2.4	1.17	1.2
Drawer	1.2	1.17	0.24

[1] The height represents the stacked transport modules on open-bed trailers, which have no roof.

Figure 2. Weather-dependent trailer configurations employed by companies. **A**. Open, used for hot and mild seasons; **B.** Single board, used when transitioning between mild and winter seasons; **C**. Double board used in winter seasons; **D**. Plastic wrap on double board modules, used in winter days or nights with extremely cold conditions.

2.2. Trailer Setup by Industry Practices

During the road transport, broilers were exposed to passive ventilation. Company live haul personnel used different management practices to mitigate seasonal impact on broilers. In mild and hot seasons, trailer modules were completely exposed to the weather ("Open", Figure 2A). In extremely cold conditions, fiberglass panels were screwed onto the exterior sides of the modules to reduce wind (called Wind board). The size of the wind board was designed to leave gaps on all edges of the module so that air can penetrate through the wires. Wind boards were installed progressively, first to cover approximately half of the exterior area of sides on each module in the fall season (refers to "Single board", Figure 2B), followed by covering about 90% of the area during the winter months ("Double board", Figure 2C). Once boards were on, they became permanent for the season. The boards were uninstalled in reverse fashion in the spring. Under extremely cold conditions or when precipitation coincided with low temperatures, some companies wrapped double-board modules using thin plastic film (70 gauge, 50.6 cm wide) immediately after birds were loaded into a module ("Plastic wrap", Figure 2D). This treatment was not fixed to the modules like the boards, allowing the integrator to select specific loads to apply the wrap to. The wrap was applied horizontally, leaving the solid top and bottom of the module uncovered by the wrap. The module was individually wrapped inside the broiler house before it was moved onto the trailer by a fork-lift. Special care was taken during the loading of the modules to avoid any edges of adjacent modules touching that could result in cuts in the wrap. Wrapping all modules for a trailer required an additional 30 min.

In the summer months, companies employed convective and evaporative cooling, i.e., fans and misting systems, to assist loading on farms during the daytime. In general, a fan assembly, consisting of a single or a linear array of multiple propeller fans spaced evenly, was positioned close to the transport trailer, and blows air into one side of it ("Cooling Assist"), while catching personnel brought modules with birds from a house and stacked them on the trailer from the opposite side. Water from misters on the fan assembly was blown toward the trailer under loading. Occasionally, a hand-held pressure washer attached to a house faucet was operated to apply water to the trailer during loading.

2.3. Data Collection of Live Haul Trips

The data were collected from six broiler processing plants of four companies from 2015 to 2016. Catching personnel loaded broilers into the modules. The majority of the cold-season monitoring trips were conducted at two processing plants slaughtering small-size broilers (nominal weight of 1.7 kg). Loading densities ranged from 34 to 36 birds per drawer (with floor space of 1.35 m^2 per drawer), or 43 to 45 kg m^{-2}. Warm-season monitoring trips were conducted at four processing plants slaughtering medium-size broilers (nominal weight of 3.0 kg). Loading densities ranged from 20 to 22 birds per drawer, or 44 to 49 kg m^{-2}. Monitoring occurred during the daytime or nighttime in all seasons. The test procedures of this study were approved by the Institutional Animal Use and Care Committee of the University of Arkansas under Protocol # 15026.

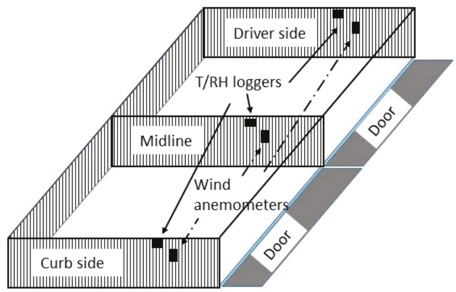
Trailer boarding or cooling assistance were applied at the discretion of the live haul personnel. Thirty-three trips were monitored in total, with journey time ranging from 15 to 125 min (median of 60 min). The monitored trip lengths may not represent the typical journey lengths in this concentrated broiler production area, since we intentionally selected longer journeys to monitor due to the perceived potential challenges faced by broilers during long hauls. Journeys shorter than 40 min or with partial trailer loads were excluded from this report, leaving 28 trips for further analysis (Table 2). On two separate winter days, i.e., 11 January 2016 & 19 December 2016, two trailers either double board or plastic wrapped were instrumented identically and moved chickens from the same farm to the same plant at night; this allowed us to compare impact of wrapping on the thermal environment.

Table 2. Range of ambient temperatures of trailer configuration for 28 trips and their transit duration.

Ambient Temperature (°C)	Trailer Configuration		No. of Trips	Transit Duration, min
	Boarding	Cooling Assist at Loading		
−15.8 to 2.8	Plastic wrapped		5	75 to 125
−16.4 to 8.9	90% boarded		4	78 to 107
2.3 to 20.0	Half boarded		7	41 to 84
12.2 to 29.4			4	63 to 105
29.3 to 36.1		Fan + water	8	42 to 74

2.4. Instrumentation

Temperature and relative humidity (RH) (Hobo U23 Pro v2, −40 °C to 70 °C, ± 0.2 °C, and 0 to 100%, ± 2.5%, Onset Computer Corporation, Bourne, MA, USA) were recorded continuously at 25 locations throughout the trailer with T recorded at additional 20 locations (DS1921L Thermochron, ± 0.5 °C, Maxim Integrated, Sunnyvale, CA, USA). Air speed were measured by six anemometers (Kestrel 4000; accuracy ± 0.1 m s^{-1} from 0.4 to 40 m s^{-1}, Minneapolis, MN, USA) on two modules. Before each scheduled monitoring campaign, data loggers were installed at pre-determined module locations on empty trailers using cable ties at the processing plant (Figure 3a,b). Nine thermal loggers occupied each of the five vertical cross-sections (Figure 4). Temperature, humidity and wind were measured every 30 s. Times of departure from farm to the processing plant were recorded using a GPS unit (eTrex 20, Garmin, Olathe, KS, USA) that logged waypoints every second, downloaded after each trip.



a b

Figure 3. (**a**) Thermal and wind dataloggers secured on curbside of a transport module during a monitoring campaign. (**b**) Diagram of instrumented transport drawers showing loggers on curb side, midline and driver side. The interior loggers consist of those on the midline, while exterior loggers consist of those on driver and curb sides.

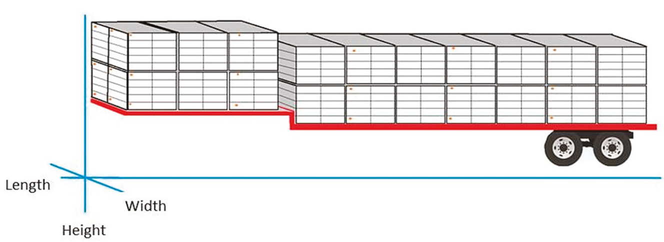


Figure 4. Schematic of an industry-standard drop deck poultry trailer loaded with 20, 10-door modules. Dataloggers (shown as orange dots) for temperature were installed as a 5 × 3 × 3 grid on the trailer. Three horizontal planes along height are Top, Middle, and Bottom. Three longitudinal planes along width are Driver, Midline and Curb.

2.5. Data Analysis

Dry-bulb (T_{db}), dew point temperatures (T_{dp}) and relative humidity data were downloaded from the data loggers after each monitoring campaign. Ambient T and humidity corresponding to the monitored duration of each trip were obtained from weather data downloaded from nearby weather stations on the days that monitored trips took place.

Apparent equivalent temperature (AET) were calculated from the dry bulb temperature and the relative humidity [17]. Specifically,

$$AET = T + \frac{10^{\left(30.5905 - 8.2 \times \log_{10}(K) - \frac{3142.31}{K}\right)} \times \left(\frac{RH}{100}\right)}{0.93 \times (0.0006363601 \times K + 0.472)} \quad (1)$$

where T = recorded air temperature, °C, K = T corrected to Kelvin (°C + 273.15), RH = recorded relative humidity, %.

Except for mean comparison of recorded temperature, elevated temperature above ambient values (i.e., Δt) were calculated by subtracting mean ambient temperature of each transit from the recorded temperature at each measurement location. For each management configuration, elevated temperature at three longitudinal planes (width, i.e., driver, midline and curb), at three horizontal planes (height, i.e., top, middle and bottom), and at five cross-section planes (Figure 3) were analyzed by ANOVA with means separated by Tukey's range test [18].

Mean comparison of elevated temperature of different transit segments at three longitudinal and three horizontal planes of plastic wrap and double board were made [18]. The first segment was chosen as 15-min, due to an observed fast change immediately after the trailers departed from the farms, especially in summer months. Other segments were 30-min long. Only data from four segments were retained for this analysis. Differences of segment-average temperature were analyzed within groupings for each plane along the width and height axes [18], and considered significant if $p < 0.05$. Additionally, mean comparisons of elevated temperature above ambient (Δt) at different planes of the two paired trailers with either plastic wrapped or double board were made using paired t-test to determine the effect of plastic wrap on the double board winter trips.

Relative humidity values represent how close air is to saturation at the measured temperature. Due to its temperature dependence, it is invalid to compute averages of recorded relative humidity over several hours directly. A "representative" relative humidity variable (RH*) was derived from a time-averaged humidity ratio (also called absolute humidity) and a corresponding time-averaged temperature from the same logging interval [19]. It serves as an "averaging" variable of relative humidity using appropriate psychometric manipulation.

Specifically, for each data logger, a humidity ratio (W) was computed at a specific time using its corresponding T and RH values [20]. After calculating the humidity ratio for a x time interval, a time-averaged humidity ratio ($\overline{W}$) and a time-average temperature ($\overline{T}$) were calculated for this duration. Before the representative RH* can be calculated, the partial pressure of water vapor in moist air (p_w) was calculated using:

$$p_w = \frac{\overline{W} \times p}{(\overline{W} + 0.62198)} \quad (2)$$

where p_w = partial pressure of water vapor in moist air (Pa), $\overline{W}$ = time-averaged humidity ratio, p = total pressure of moist air assumed equal to atmospheric pressure (Pa).

The representative relative humidity was determined by:

$$RH^* = \frac{p_W}{p_{WS}} \times 100 \quad (3)$$

where RH* = representative relative humidity (%), p_{ws} = saturation pressure of moist air (Pa).

3. Results and Discussion

Figure 5 illustrates examples of the temperature, RH and air speed profiles of interior and exterior of a summer live-haul trip. The ambient temperature at the start of this transport was 30.6 °C (Figure 5a). The first arrow indicates the beginning of loading, the second the beginning of transport, and the third the arrival at the processing plant for holding period. Cooling, including convective fans and water sprays, was applied to the trailer during loading, resulting in a sharp temperature drop and relative humidity increase. Air speeds during the 40-min transport were variable (Figure 5b), likely in part determined by the speed of the vehicle. Air speeds at interior and exterior locations averaged 0.5 and 1.9 m s^{-1}, respectively. Webster et al. [5] reported mean air movement of open trucks in motion of 3.3 m s^{-1} (range 0.0 to 8.9 m s^{-1}) of commercial broiler transporters in England. Weeks et al. [10] calculated that air speeds in moving vehicles varied from 0.9 to 2.4 m s^{-1} with maxima of 6.0 m s^{-1}.

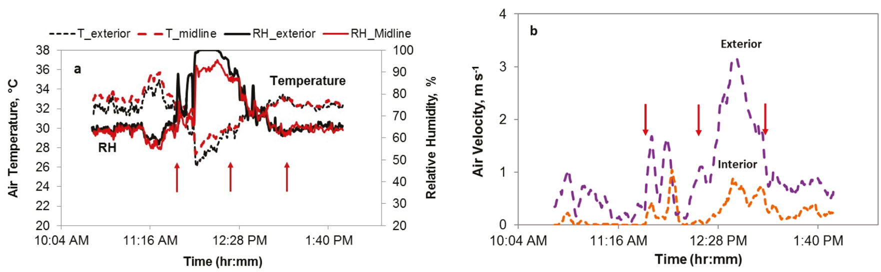


Figure 5. Temperature, relative humidity (**a**) and air velocity (**b**) profiles from interior and exterior logger positions of a summer trip. The first arrow on each graph indicates the beginning of loading, the second the beginning of transport, and the third the beginning of holding period.

The representative relative humidity of 28 trips and their trailer average temperatures are plotted in Figure 6. Using a physiological stress response model, Mitchell et al. [17] identified "safe", "alert" and "danger" thermal zones, defined by AET values based on temperature-humidity combinations (Figure 1). AETs of 65 °C or greater were deemed dangerous due to potential severe physiological stress [17]. AETs of the monitored summer trips averaged 80.5 °C, indicating possible dangerous thermal conditions. Note that the laboratory experiments used to collect physiological parameters for derivation of the AET index were three hours in length with no air speed reported [17]. Majority of transport trips in this study area were less than two hours, with a median of one hour. Air speed on the moving trailers (Figure 5b) may have allowed convective cooling, although this was not uniformly experienced by all chickens on board. Additionally, partial surface wetting of broilers by hand sprayers may have alleviated or delayed the onset of heat stress of cooling-assisted transport trips based on literature reports [21,22].

The severity of physiological stress in the summer is unknown due to unavailable mortality data, which could have allowed correlation analysis with the thermal conditions. Future research should focus on improved research protocols, such as mortality data collection, measurements of core body temperature of broilers under various micro-environments, and behavior monitoring with video cameras. Nevertheless, it is important for commercial companies in South Central USA to improve the efficiency of the catching, loading and transporting process, and to minimize the duration of exposure of live chickens to uncontrolled environments in the summer. Additionally, better measures, such as stocking density adjustments, route optimization to avoid heavy traffic, and adding on-board sprinkler systems to the modules, should be considered for trips with longer distances to mitigate heat stress conditions.

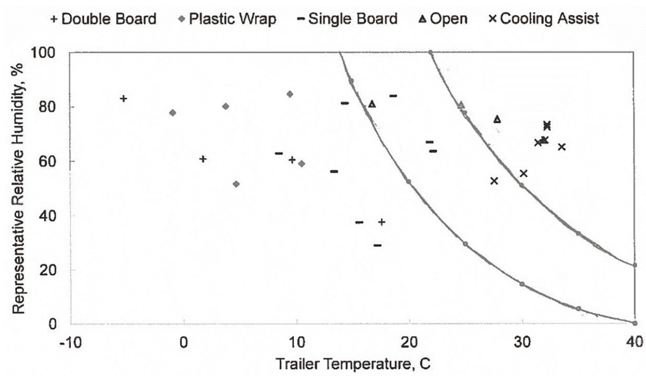


Figure 6. Average representative relative humidity vs. average trailer temperature of 28 monitored trips moving broilers to processing plants. The AET values corresponding to 40 and 65 °C [17] were overlaid.

Tables 3 and 4 show the mean temperature and representative relative humidity that birds were exposed to for every 15 or 30-min transit duration of each trailer configuration. Under winter conditions, double boards and plastic wraps allowed heat produced by the broilers to be retained within the transporters, resulting in a mean trailer temperature of 5.5 °C (Table 3). While recorded trailer temperatures were higher than ambient T in winter (Table 3), they remained below the thermoneutral range [10], in spite of the boarding procedures to ameliorate the cold temperatures. Knezacek et al. [6] reported a 1 °C rectal temperature reduction of broilers that were exposed to 3.9 °C crate temperature during a 178-min winter trip of −28.2 °C ambient temperature. In a simulated 3-h wind tunnel experiment with chamber temperatures ranging between −4 and 12 °C, 1 °C rectal temperature reduction from broilers were reported under all dry chamber environments [23]. However, moderate to severe hypothermia (3 to 14 °C rectal temperature reduction) were observed when wetting was imposed in an increasingly colder chamber environment [23].

Table 3. Mean recorded temperature (°C) of 15 or 30-min segments during transit under five trailer configurations found in commercial broiler transporters.

Duration	Plastic Wrap	Double Board	Single Board	Open	Cooling Assist
Ambient	−5.3 ± 3.2	−3. 5± 5.4	10.0 ± 1.7	22.0 ± 6.3	31.5 ± 3.0
Transit:					
0–15 min	6.9 ± 2.6	6.6 ± 2.9	16.9 ± 2.1	25.0 ± 5.4	29.6 ± 2.2
15–45 min	6.2 ± 2.7	6.2 ± 3.1	16.6 ± 2.2	25.3 ± 5.6	32.1 ± 1.9
45–75 min	5.1 ± 3.2	5.8 ± 3.6	18.5 ± 3.5	25.2 ± 6.8	33.1 ± 1.1
75–05 min	3.7 ± 6.0	6.1 ± 5.7			

Table 4. Mean representative relative humidity (RH*, %) every 15 or 30-min during transit under five trailer configurations found in commercial broiler transporters.

Duration	Plastic Wrap	Double Board	Single Board	Open	Cooling Assist
Ambient	68.1 ± 6.8	71.5 ± 4.3	62.4 ± 10.0	82.4 ± 5.4	51.8 ± 4.2
Transit					
0–15 min	78.7 ± 2.8	70.6 ± 8.5	55.4 ± 7.4	76.7 ± 2.4	72.5 ± 4.3
15–45 min	74.7 ± 3.6	68.5 ± 6.7	56.3 ± 8.8	66.9 ± 1.8	70.4 ± 4.7
45–75 min	73.7 ± 4.6	67.6 ± 7.1	35.1 ± 8.8	64.2 ± 0.0	70.8 ± 6.3
75–105 min	74.5 ± 10.4	56.7 ± 9.3			

During the warm season with cooling assistance, overall trailer temperatures were within a narrow range of ambient conditions (up to 36.1 °C) for the duration of the journey (Table 3), with mean representative relative humidity less than 80% (Table 4). Both the air speed on trailers (Figure 5b) and the water retained by birds and trailer modules during on-farm loading could have prevented trailer air temperatures from rising far beyond ambient temperatures. Panting was observed from birds in transit based on camera footages from one selected summer trip, indicating that the efficacy of cooling assistance was limited due to a simultaneous increase of humidity level.

Temperature throughout transporters with open and single side boards were mostly within the thermoneutral zone for broiler chickens (Table 3). Open transporters operating in the mild seasons provided a reasonably comfortable thermal environment. It is important to note that the greatest number of broilers are transported using the open sided configuration.

Humidity plays an important role in heat and mass exchanges in the livestock and poultry environment [4,15]. For example, moist air can compromise feather insulation properties, placing broilers at risk of cold stress [8,23]. Hunter et al. [23] concluded that broiler chickens could be safely transported at crate temperatures as low as −4 °C, if they are dry, or experience moderate hypothermia at temperatures as high as 8 °C when wet.

Representative relative humidity was selected to express the extent of moisture saturation in the modules. When moist air comes into contact with cooler surfaces (i.e., the modules and interior surface of wrapping plastics), condensation forms. Burlinguette et al. [8] used a threshold of 80% relative humidity value to determine susceptibility to condensation. In our study, mean representative relative humidity of four winter boarded transport was around 80% (Figure 6), indicating that a small amount of air exchange existed. Movement-induced ventilation prevented the excessive accumulation of moisture produced by the birds within the trailers (Table 4).

3.1. Spatial Uniformity of Air Temperature on Trailer

The industry practice of installing fiberglass boards on the modules is intended to reduce ventilation and conserve heat produced by the broilers in cool seasons. The practice seemed to be effective, elevating mean air temperature above their corresponding ambient temperature (with ranges of −15.8 to 2.8 °C and −16.4 to 8.9 °C, Table 2) by 10.7 °C and 9.3 °C for the plastic wrap and double board, respectively (Table 5). In comparison, three levels of curtains, and closure of roof vents used by Canadian transporters, resulted in an average temperature elevation of 14.4, 12.7 and 11.2 °C above ambient, as reported by Burlinguette et al. [8].

Differences in elevated temperature above ambient between locations were analyzed for each configuration. In winter, elevated temperatures at three longitudinal planes along the Width axis (Table 5) were different ($p < 0.05$) for all three boarding configurations. Mean elevated T at Midline were warmer than those at the outward-facing planes of the trailers ($p < 0.05$) when side boards were used, likely a result of lower airflow in the central locations. Top modules recorded mean T elevations of 8 °C from the ambient, which were several degrees lower than those gained by the middle or bottom modules, likely due to lack of protection from motion-induced ventilation. The lowest observed T was −1.1 °C at top, curb-side module, while the highest T, 18 °C, occurred on the midline, bottom module on double board trailers. This was similar to earlier report of highly variable and extreme thermal conditions when side curtains and most roof vents were closed on a Canadian transporter [8]. Large temperature gradients with up to 20 °C difference of crate temperatures (i.e., 3 to 26 °C) in an ambient temperature of −28 °C were reported when only the fourth roof vent was opened in a 178-min trip on a Saskatchewan transport trailer [6]. Kettlewell et al. [1] reported airflow movement from the back to the front of the trailers based on the temperature trends observed throughout trailers in the UK. However, the airflow distribution of the trailers in this study is unknown due to many undefined small openings at the back of each module (opposite to the door) (Figure 3b) and around the fiberglass boards.

Table 5. Spatial variation of air temperatures elevation above-ambient (Δt, °C) across the trailer during transport in different seasons.

Location	Plastic Wrap	Double Board	Single Board	Open	Cooling Assist
Width					
Driver	9.9 ± 1.3 ^{b}	8.1 ± 0.6 ^{b}	4.8 ± 0.8 ^{c}	4.1 ± 1.5 ^{c}	−0.10 ± 1.0 ^{c}
Midline	11.9 ± 1.3 ^{a}	11.5 ± 0.6 ^{a}	6.5 ± 0.8 ^{a}	5.2 ± 1.5 ^{a}	0.78 ± 1.0 ^{a}
Curb	10.4 ± 1.3 ^{b}	8.4 ± 0.6 ^{b}	5.5 ± 0.8 ^{b}	4.6 ± 1.5 ^{b}	0.35± 1.0 ^{b}
Height					
Top	8.7 ± 2.4 ^{c}	8.2 ± 1.2 ^{b}	5.4 ± 1.4 ^{a}	4.6 ± 2.7 ^{b}	1.7 ± 1.0 ^{a}
Middle	13.1 ± 2.4 ^{a}	9.8 ± 1.2 ^{a}	5.6 ± 1.4 ^{a}	4.5 ± 2.7 ^{ab}	−0.20 ± 1.0 ^{b}
Bottom	10.4 ± 2.4 ^{b}	10.1 ± 1.2 ^{a}	5.8 ± 1.4 ^{a}	4.8 ± 2.7 ^{a}	−0.03 ± 1.0 ^{b}
Length [1]					
1	10.0 ± 1.4 ^{bc}	10.5 ± 0.7 ^{a}	6.3 ± 0.8 ^{a}	5.3 ± 1.5 ^{a}	0.54 ± 1.0 ^{a}
2	10.6 ± 1.4 ^{b}	8.5 ± 0.7 ^{c}	4.7 ± 0.8 ^{b}	4.5 ± 1.5 ^{b}	0.46 ± 1.0 ^{ab}
3	13.5 ± 1.4 ^{a}	10.4 ± 0.7 ^{a}	6.5 ± 0.8 ^{a}	4.9 ± 1.5 ^{c}	0.46 ± 1.0 ^{ab}
4	9.8 ± 1.4 ^{bc}	9.4 ± 0.7 ^{b}	5.2 ± 0.8 ^{b}	4.4 ± 1.5 ^{c}	0.18 ± 1.0 ^{ab}
5	9.7 ± 1.4 ^{c}	8.0 ± 0.7 ^{c}	5.2 ± 0.8 ^{b}	4.0 ± 1.5 ^{d}	0.08 ± 1.0 ^{b}

^{a,b,c} Superscripts denote differences ($p < 0.05$) within each column and axis, [1] Code 1, 2, 3, 4 and 5 denote instrumented cross sections from the front to the back of trailers.

Transporters using cooling assistance displayed slightly different temperature profiles than those during cold or mild seasons. Temperatures tend to be higher ($p < 0.05$) at the midline (Table 5), although the difference was small (1.0 °C). The top tier displayed higher temperature elevations ($p < 0.05$), likely due to exposure of sheet metal roof to direct sunlight in summer.

3.2. Effect of Journey Length on Thermal micro-Environment

Although journey lengths in this study were shorter (less than 2 h) compared to those reported elsewhere [1,6,10,19], elevated temperatures still differed from the beginning to the end ($p < 0.05$) (Table 6) for trips using plastic wrap. Trailer T elevation decreased significantly during transit at various locations in plastic wrap (up to 4.1 °C). A similar decline of elevated temperature above the ambient were observed in double board trailers without wrap (Table 7).

Table 6. The effect of trip duration on elevated temperature above ambient (Δt, °C) at measured locations across width and height of the trailer with plastic wrap (n = 5).

Duration	Width			Height		
	Driver	Midline	Curb	Top	Middle	Bottom
0–15 min	11.3 ^{a}	13.8 ^{a}	11.7 ^{a}	10.9 ^{a}	14.7 ^{a}	11.1 ^{a}
15–45 min	10.6 ^{ab}	12.6 ^{a}	11.2 ^{ab}	9.2 ^{b}	14.1 ^{a}	11.2 ^{a}
45–75 min	9.8 ^{b}	11.5 ^{b}	10.2 ^{bc}	8.3 ^{b}	12.7 ^{b}	10.5 ^{a}
75–105 min	8.0 ^{c}	9.7 ^{c}	8.6 ^{d}	6.6 ^{c}	10.9 ^{c}	8.7 ^{b}

^{a,b,c,d} Superscripts denote differences ($p < 0.05$) within each column and axis.

Table 7. Effect of trip duration on elevated temperature above ambient (Δt, °C) at measured locations across the width and height of the trailer with double boards (n = 4).

Duration	Width			Height		
	Driver	Midline	Curb	Top	Middle	Bottom
0–15 min	10.1 ^{a}	12.6 ^{a}	10.0 ^{a}	9.6 ^{a}	11.5 ^{a}	11.6 ^{a}
15–45 min	8.8 ^{b}	12.7 ^{a}	8.9 ^{ab}	8.8 ^{a}	10.8 ^{a}	10.8 ^{ab}
45–75 min	7.8 ^{c}	11.5 ^{b}	8.2 ^{bc}	7.9 ^{b}	9.6 ^{b}	10.0 ^{bc}
75–105 min	5.9 ^{d}	9.0 ^{c}	6.7 ^{d}	6.3 ^{c}	7.3 ^{c}	8.0 ^{d}

^{a,b,c,d} Superscripts denote differences ($p < 0.05$) within each column and axis.

When cooling assistance was used during loading in summer, temperatures increased from the first 15 min to the following 30 min across the trailer ($p < 0.05$) (Table 8). Air temperatures inside the trailers were lower than the corresponding outdoor conditions during the first 15 min immediately after trailers departed from farms. This was clearly the residual effect of liquid water retained on transporters from loading on farms. Fans and various types of water treatments, including misters and hand-held sprayers, were used in all eight trips monitored and reported in this category. Water retained by modules and birds' feathers continued evaporating as transporters traveled on the roads. Ritz et al. [13] also reported that the use of multiple high-velocity fans positioned parallel to the live-haul trailers during loading was effective at cooling birds prior to transport. However, water likely diminished around 15 min after transporters' departure, allowing temperature rises of 2 to 3 °C at various locations after one hour ($p < 0.05$, Table 8). For hot weather conditions, even with 1 to 2 °C temperature rises within the trailer, thermal load could shift to a more dangerous level.

Table 8. Effect of trip duration on elevated temperature above ambient (Δt, °C) at measured locations across width and height of the trailer when cooling assistance was used (n = 8). Cooling assistance consisted of propeller fan(s) blowing air and misters or hand-held pressure washers applying water toward trailers being loaded.

Duration	Width			Height		
	Driver	Midline	Curb	Top	Middle	Bottom
0–15 min	-2.2^{b}	-0.3^{b}	-1.6^{b}	-0.1^{c}	-2.0^{b}	-2.0^{b}
15–45 min	0.8^{a}	1.4^{a}	1.3^{a}	2.4^{a}	0.4^{a}	0.7^{a}
45–75 min	1.2^{a}	1.2^{a}	1.4^{a}	1.5^{b}	1.0^{a}	1.2^{a}

^{a,b,c} Superscripts denote differences ($p < 0.05$) within each column of width and height axis.

3.3. Effect of Plastic Wrappping on the Micro-Environment

Plastic wrap, in addition to the double boarded trailers, raised the mean air temperature by around 3.2 °C compared to double boarded trailers on winter nights with average ambient temperatures of −5 and −17 °C (Table 9). Average representative relative humidities of double board and plastic wrapped trailers were 72% and 79%, respectively (Figure 6). Plastic wrapping was only used to further reduce wind penetration through modules in order to protect birds from extremely cold weather conditions when birds with incomplete feather coverage (with 1.7 kg live weight) were transported. This practice seemed to moderately retain heat and water vapor inside the modules without creating moisture saturation. Better protection, such as more insulation, might be needed in order to alleviate cold stress without any risk of creating saturated air conditions.

Table 9. Means and standard errors of elevated temperatures above ambient (Δt, °C and the paired sample t-test at various locations of paired trailers with either plastic wrap or double board on two winter nights with average ambient temperatures of −5 and −17 °C, respectively.

Treatment	Width			Height		
	Driver	Midline	Curb	Top	Middle	Bottom
Plastic wrap	12.3^{a}	14.6^{a}	11.7^{a}	10.5^{a}	16.0^{a}	11.9^{a}
Double board	8.6^{b}	11.8^{b}	8.4^{b}	8.0^{b}	10.8^{b}	10.3^{b}
Stderr	0.10	0.12	0.12	0.06	0.13	0.11
t-value	40.0	24.3	27.3	14.7	39.5	39.4

^{a,b} Superscripts denote differences ($p < 0.05$) within each column.

4. Conclusions

Temperature and relative humidity were monitored in 45 locations on 28 commercial trips hauling market-size broilers to processing plants. Weather-dependent management employed by companies, including side boards attached on the open area of modules in winter and fan trailers with mists used during loading in summer, were analyzed for their effect on altering micro-environment of the trailers. During cold weather transport when ambient temperatures were below 0 °C, on-board temperatures were lower near the exterior than in the middle, and decreased steadily as transport duration increased. Trailer temperatures on double board trailers in winter averaged 8 °C above ambient T. During warm weather transport, on-board temperatures were within ± 2 °C of the ambient, and higher near the top module of the trailers. Temperatures throughout the trailer increased by 1 to 3 °C as transit time increased in summer. Apparent equivalent temperatures of the monitored summer trips averaged 80.5 °C, indicating possible heat stress conditions based on literature reported index values. Improvement in equipment or transport management would therefore be necessary during extremely cold or hot weather.

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Factors Affecting Trailer Thermal Environment Experienced by Market Pigs Transported in the US

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Simple Summary: Transport conditions can be a challenge for pigs being transported to market. In this study, 40 trips of commercial market pigs transported from the farms to an abattoir were monitored for thermal conditions including temperature and relative humidity in order to better understand thermal variability within the trailer during transport. Variation in thermal environment inside the pig transport trailer was used as an indicator of ventilation pattern during various weather conditions. During cold weather, the front top and bottom zones were warmer than in the rest of the trailer, indicating less ventilation toward the front of the trailer. Conditions were more uniform throughout the trailer for hot temperatures, indicating sufficient ventilation to limit temperature rise. Misting showed the potential to alleviate high temperatures, but resulted in higher THI conditions. No effect of boarding and bedding combination was observed for spatial distribution of trailer interior temperatures.

Abstract: Extreme weather conditions challenge pig thermoregulation during transport and are addressed by the National Pork Board (NPB) Transport Quality Assurance® (TQA) program that provides guidelines for trailer boarding, bedding, and misting. These guidelines are widely applied, yet very little is known about the microenvironment within the trailer. In this study, TQA guidelines (V4) were evaluated via extensive thermal environment measurements during transport in order to evaluate spatial variability and implications on ventilation pattern. Effects of trailer management strategies including bedding, boarding, and misting were examined and the trailer was monitored for interior temperature rise and THI responses within six separate zones. The trailer thermal environment was not uniformly distributed in the colder trips with the top front and bottom zones were the warmest, indicating these zones had the majority of outlet openings and experienced air with accumulated sensible and latent heat of the pigs. Relatively enhanced thermal environment uniformity was observed during hot trips, suggesting that ventilation patterns and ventilation rate were different for colder vs. warmer weather conditions. Misting applied prior to transport cooled interior air temperature, but also created high THI conditions in some cases. Neither boarding and bedding combinations in the TQA nor boarding position showed impacts on trailer interior temperature rise or spatial distribution of temperature inside the trailer.

Keywords: environment; swine; transport quality; temperature; THI; ventilation; bedding; boarding; misting

1. Introduction

Road transport is a critical factor affecting pig welfare in modern commercial pork production and has been reported to increase the number of dead or down (DOD) pigs following transport as the outdoor temperature moves towards extreme hot or cold [1–11]. Despite the understanding of the underlying thermal environmental mechanisms responsible for heat- or cold-stressed pigs, challenging environments are still observed during transport in extreme weather conditions. Limiting the occurrence of poor thermal environments during transport is challenging as current trailer designs provide limited opportunity for modifying internal temperature, humidity, and air velocity [12–15].

The thermal environment in typical U.S. commercial trailers is not actively controlled, and is affected by many factors, including outdoor temperature, ventilation rate, occupant and bedding sensible and latent heat contributions, pig spatial density, trailer design and boarding management, and transport duration, resulting in conditions that are sometimes undesirable [1–4,6–13,16–19]. To address the thermal extremes that may cause distress including dead or down pigs during or after transport, the National Pork Board (NPB) developed and implemented an industry certified program, Transport Quality Assurance® (TQA), to ensure that transported pigs receive a high standard of trailer management to potentially improve trailer thermal environment [20].

The TQA guidelines include recommendations for trailer boarding (the amount of covering of trailer openings) and bedding (presence and depth of a substrate such as wood shavings) that vary with outdoor temperature [14,15,20]. Changes in boarding, in principle, result in changes in net ventilation of the trailer during transport, although variations in boarding patterns (openings toward the front, or the rear, or uniformly along the trailer sides) are not addressed in the TQA. Bedding provides potential insulative effects for the pigs during cold weather and increases footing for the pigs while moving into and out of the trailer, although the likelihood of frozen bedding during extreme cold weather exists [14,15].

Industry implementation of TQA has significantly reduced the number of dead or fatigued pigs at arrival at processing facilities [21]. Past TQA recommendations for bedding, boarding and misting allow for some management practices to vary among producers [20]. For example, under ideal conditions, evaporative cooling has been shown to relieve heat stress conditions and may be achieved by sprinkling pigs and bedding when increased air velocity is provided by fans or during transport [22–27]. Alternatively, air cooling by fogging has also shown relief of heat stress conditions by lowering the air temperature. TQA guidelines do not distinguish between these two wetting techniques or provide guidance for how to implement either.

Further exploration of trailer thermal environment under TQA guidelines is merited to better understand the factors contributing to undesirable conditions. Projects commissioned by the NPB to evaluate and revise TQA guidelines found that the guidelines required minimal changes [28–34]. It was reported by [30] that the highest rate of DOD pigs at arrival occurred when low boarding (<30% coverage) was used for outside temperature < 5 °C. It was found by Kephart et al. and McGlone et al. [31,32] that adding more than 6 bales of bedding did not provide benefits to the pigs, nor did it worsen mortality or morbidity rate during cold weather (<10 °C); however, bedding in excess of 3 bales/trailer during warm weather (>21 °C) showed negative impacts for DOD on arrival. It was found by Kephart et al. and McGlone et al. [33,34] that three methods of sprinkling (on pigs only, on bedding only, or on pigs and bedding) did not have effects for pig measures including pig surface temperature, vocalizations, slips and falls, or transport DOD losses but did show increased stress signs for pigs. The results summarized to date included pig or transport loss measures at the abattoir and averaged or generalized temperatures, rather than comprehensive dynamic measurements of thermal environment during transport. One possible factor affecting the number of DOD pigs is the microenvironment that pigs experience within a trailer. An analysis focused on investigating the variability within the trailer has the potential to identify some conditions that might pose a challenge to a subset of the pigs during transport.

The observational study reported in this paper builds on findings in a companion paper by Xiong et al. [15], where an instrumentation system was designed and implemented into a newly fabricated commercial pig trailer and used throughout the entire study [14,15]. The previous report included 31 trips, with thermal comfort classified as extreme cold, cold, thermoneutral, warm, hot, or extreme hot, based on trailer interior temperatures measured, and did not examine effects of trailer boarding, bedding, or misting on trailer interior temperatures, or explicitly account for the outside temperatures during each trip. The previous paper [15] presented an overview of observations from 31 trips that fully complied with TQA V4, and noted that pigs experienced undesirable thermal conditions for outside temperature below 5 °C or above 27 °C. A Livestock Weather Safety Index in the emergency heat stress category was observed in the trailer when outside temperature exceeded 10 °C. Trailer rear zones most frequently resulted in maximum pig surface temperatures, and middle zones most frequently resulted in minimum pig surface temperatures. Varying boarding levels and distributions showed the potential for altering the ventilation patterns within the trailer and merited further exploration as a technique to increase thermal uniformity throughout the trailer by manipulating the location of fresh air inlets and outlets. This paper provides a more detailed analysis of transport trailer microenvironment to understand spatial variability within the trailer and includes a total of 40 trips. Specifically, this study addresses two objectives:

(1) to assess the spatial variation of the thermal environment in the trailer during 40 commercial transport trips of market-weight pigs under different weather conditions;
(2) to evaluate the effects of trailer management methods including bedding and boarding for cold weather, and misting strategy for hot weather, on trailer interior temperature and temperature and humidity index (THI).

2. Materials and Methods

2.1. General Information

This paper summarizes results from a multi-year commissioned study that involved three to five monitoring trips every month for over a year, with each monitoring covering a complete transport trip for market-weight pigs (from loading pigs at a pig barn to finish unloading at an abattoir) in the Midwestern U.S. The objective of the commissioned study was to evaluate effects of industry implementation of trailer management (bedding, boarding, and wetting) on trailer interior thermal environment as outlined in the TQA handbook [20]. Therefore, the field measurement was dictated by the outdoor conditions and the specifications of the TQA guidelines. The detailed information of the trailer description, instrumentation of the monitoring system, analysis of pig surface temperature, pig mortality on arrival, effects of trailer bedding depth and boarding percentage on pig skin surface temperature during cold and mild weather conditions are found in the companion paper that characterizes the observations over all weather conditions [15].

2.2. Trailer Description and Measurement System Overview

A newly fabricated commercial double-decked pot-belly livestock transport trailer designated for pig transport was used in this study (Figure 1). While this style trailer can be converted to a three-deck configuration for transporting weaned piglets, the trailer in this study was used with only two decks for market pigs throughout the study. The trailer was equipped with three hinged gates on each deck and a loading ramp deployed at the bottom rear. Four 25 × 25 cm nose vents are located in the front corners of the trailer (two to a side, as shown in Figure 1). All nose vents are completely open during hot weather and completely covered during winter. The trailer was divided into six animal zones, numbered from 1 to 3 on the top and 4 to 6 on the bottom deck, from the front to the back. A monitoring system was developed to measure thermal conditions inside each of the six trailer zones. A detailed trailer schematic with zone compartmentalization and monitoring system can be accessed from the companion paper from the same study [15]. The monitoring system in each zone consisted of 14 thermistors (Model 10M5351, Honeywell Parts, Phoenix, AZ, USA), for a total of 84 thermistors in the trailer, to measure pig-level air temperature and one centrally-located temperature and relative humidity (RH) probe (Vaisala INTERCAP HMP60, Vaisala, Vantaa, Finland) per zone to capture the center-zone condition near the ceiling. A weather station was installed outside of the trailer to capture the outside temperature (T_{out}) and RH. Data was recorded every minute.

Figure 1. Trailer used for trailer environment monitoring in this field study. The same trailer was instrumented and utilized for all monitoring of trailer interior environment during all commercial market-weight pigs transport throughout this study.

2.3. Procedures during a Commercial Pig Transport Trip

During a typical commercial pig transport trip in the Midwestern U.S., the trip generally proceeds in the following segments: (1) arriving at a commercial pig barn and loading market-weight pigs, where variable waiting time at the barn can occur; (2) departing for road transport, and the duration of the transport varies greatly due to distance between scattered barn locations and the abattoir; (3) arriving at the abattoir and unloading the pigs, where waiting time may likely occur due to uncertain processing schedules at the abattoir. During summer conditions, additional cooling procedures may be additional to the trip, based on availability at the barn or abattoir. As for this study, we observed two combinations that can be flexibly available to the trailer operator, including applying misting to the trailer interior at the pig barn, and applying misting at the abattoir, with or without accessing air flows compensated by external fans.

2.4. Summary of Field-Monitoring Trips

A total of 40 commercial transport trips for market-weight pigs were successfully monitored with the instrumentation system from May 2012 to February 2013, covering a wide distribution of outdoor conditions, including extreme temperature events [14]. The same trailer was used, and the same driver was responsible for operating and configuring the trailer and managed the animals during all monitoring trips to avoid discrepancy in trailer design, management, and animal handling. All trailer procedures, including misting the interior trailer, bedding and boarding arrangements followed TQA general recommendations. Truck average velocity was approximately the same across all monitoring trips. All trips were conducted with full loading capacity (170–175 market-weight pigs at 127–136 kg each). With a 79.2 m^2 total trailer floor space, the loading density was 275–300 kg/m^2.

These 40 trips were categorized into five thermal categories based on average T_{out} recorded during each trip. Table 1 summarizes the thermal categories and the number of trips included in each category. Analyses of trailer management were broken into cold weather analysis that included trips in *Very Cold* and *Cold* categories, and hot weather analysis that included trips in *Mild*, *Warm*, and *Very Hot* categories.

Table 1. Summary of field-monitoring trips completed with thermal categories based on average outside air temperature range recorded during each trip.

Evaluation	Thermal Category	T_{out} Range (°C)	Number of Trips
Cold Weather Analysis	*Very Cold*	<−12	4 *
	Cold	−12 to 9	16
	Total cold weather trips		20
Hot Weather Analysis	*Mild*	10 to 26	8
	Warm	27 to 32	7
	Very Hot	>32	5
	Total hot weather trips		20

* For the *Very Cold* category, one trip experienced thermistor failures and was excluded from this table and analysis involving pig-level air temperatures.

2.5. Evaluation of Hot Weather Trips

2.5.1. Misting Procedure

Misting the inside of a stationary trailer and/or access to external fans when available is suggested as an option by the TQA [20] for summer conditions, although the placement and the operating pressure of nozzles are not clearly addressed and can be customized by transport companies. Misting in this study indicated spraying water into the air or onto the back of pigs and/or onto the bedding materials. For the trailer used in this study, 20 misting nozzles (TX-V626, Teejet Technologies, 2 in Zone 1, 6 in Zone 2, 2 in Zone 3, 1 in Zone 4, 6 in Zone 5, and 3 in Zone 6) were located along the middle length on the bottom level, and the right-side length on the top level. While loading pigs at commercial pig barns, two methods of misting were observed based on the water availability, including misting during the process of loading the pigs onto the trailer, or misting briefly after the trailer was fully loaded. Fan banks were located in the abattoir and were available only when the trailer was parked by the fan banks prior to unloading. The duration of misting varied among practices and usually lasted about 5 to 15 min.

2.5.2. Effects of Trailer Management Methods for Hot Weather

Temperature measurements from all of the thermistors in each zone were averaged for the segment during transport (depart from pig barn where pigs were loaded until the arrival at the abattoir). A temperature and humidity index (THI) was computed (Equation (1)) using the center-zone temperature and RH data [14,25]. The average and maximum THI inside the trailer, and the average THI for outside condition during the transport segment were obtained.

$$THI = 0.8T_{db} + RH(T_{db} - 14.4) + 46.4 \quad (1)$$

Four response variables were tested individually to assess the spatial thermal variability within the trailer during transport: (1) average temperature difference between the trailer zones and the outside condition; (2) average THI; (3) maximum THI; and (4) difference between the maximum THI recorded in the trailer and the average THI for outside condition. Each response variable was analyzed for 20 hot weather trips in thermal categories including *Warm*, *Mild*, and *Very Hot* by analysis of variance (ANOVA) for effects of thermal category, trailer zones, misting methods at loading (no misting, misting during loading process or misting after all pigs were loaded), and zone x misting interaction. The analyses were done by PROC MIXED in SAS (version 9.4, [35]). PROC UNIVARIATE was used to verify normality of the dependent variable and accepted at $p > 0.05$. The Tukey–Kramer test for differences of least square means was used to determine significant differences between variable means ($p < 0.05$) due to unequal sample sizes.

2.5.3. Temporal Thermal Profile inside the Trailer for Hot Weather

Center-zone temperatures in the six trailer zones, the outside temperature and center-zone THI conditions were plotted against elapsed time for two representative monitoring trips, including a morning trip in the *Very Hot* category and an afternoon trip in the *Warm* category. Important segments, including arriving at a pig barn, loading pigs onto the trailer, misting inside trailer, road transport, arrival at abattoir, access to fans and misting during waiting, and unloading pigs are identified. The temporal temperature profile was investigated for both hot and cold weather conditions, and the temporal THI profile was conducted for the hot weather conditions only, with one representative sample trip from each data set.

2.5.4. Spatiotemporal Visualization of Variability inside the Trailer for Hot Weather

Temperature distribution patterns on a trailer deck basis (top or bottom) provide visualization of multidimensional temporal variability within the trailer and insight into ventilation patterns during transport. Based on our understanding of the ventilation patterns, cooler regions indicate proximity to an air inlet and hotter regions indicate air outlets in the trailer, except in the case of misting or sprinkling. This is expected from the understanding of pressure distributions on the outside of a moving trailer, with lower pressure inside the trailer that drove air in.

Data from 84 pig-level temperature sensors were linearly interpolated in Matlab® to develop a series of animations that represent the dynamic spatiotemporal profile across the trailer inside [14]. The animations are interpreted as follows: red indicates warmer temperatures and blue indicates cooler temperatures; green circles represent pig-level thermistors and their locations within the trailer; and a colored text box indicates critical events which occurred during the monitoring trip. The animations subjectively describe the ventilation patterns within the trailer, areas receiving benefits from the cooling methods (including misting onto pigs and access to external fans), and the evaporative cooling persistence into the transport segment.

The spatiotemporal visualization of trailer interior temperatures was developed for both hot and cold weather conditions. Representative trips monitored during *Very Hot* (2 trips), *Warm* (1 trip), and *Mild* (1 trip) categories were selected to create the animations. Table A1 (Appendix A) provides the descriptive information for these four trips, with viewable animations available in the Supplemental Materials accompanying this article.

2.6. Evaluation of Cold Weather Trips

2.6.1. Boarding and Bedding Procedures

Trailer boarding (covering of trailer openings) was recommended in the TQA for winter conditions. The TQA guidelines outlined 25%, 50%, 75%, and 90% boarding coverage for specific winter outside temperature ranges. Three variations in boarding patterns (uniformly along the trailer sides, boarding gradually more towards rear, and boarding all at the back) are not addressed in the previous TQA guidelines and are evaluated in this study. The industry often applies boarding uniformly along the trailer side.

Bags of conventional kiln-dried pine shavings were used as bedding in this study (0.06 m^3 each). The use of bedding was characterized by the number of bags placed onto the trailer prior to each monitoring trip and was designated for specific outside temperature ranges: light bedding (1 or 2 bags); medium bedding (3 bags); and heavy bedding (4 to 6 bags). According to the TQA guidelines, the trailer operator had the flexibility to slightly adjust the number of bags of bedding within each designation [20].

2.6.2. Effects of Trailer Management Methods for Cold Weather

Trailer management methods for cold weather investigated were trailer boarding percentage, bedding level, and boarding position. A boarding-bedding combination was created for the analysis, where LM indicates light boarding (25%) and medium bedding (3 bags); MM indicates medium boarding (50%) and medium bedding; and MH indicates medium boarding and heaving bedding (4–6 bags).

The average temperature difference between the trailer zones and the outside condition was analyzed for 16 trips under T_{out} thermal category *Cold* by ANOVA for effects of trailer zones, combination of bedding and boarding percentage, boarding position (as a nesting factor in the boarding and bedding combination, including boarded evenly, more towards the rear, or all at the rear), and zone x boarding position interaction. The analysis was performed by PROC MIXED statement in SAS (version 9.4, [35]). PROC UNIVARIATE was used to verify normality of the dependent variable and accepted at $p > 0.05$. The Tukey–Kramer test for differences of least square means was used to determine significant differences between factor means ($p < 0.05$) due to unequal sample sizes.

For the four trips monitored under the *Very Cold* category, the analysis was simplified due to the lack of combinations of boarding-bedding placement and boarding position in trips monitored, and only the effect of trailer zones on trailer interior temperature rise was analyzed. The boarding-bedding combination and boarding position were not tested. A representative trip in the *Very Cold* category was selected to create the spatiotemporal animation (Table A1 (Appendix A)).

3. Results and Discussion

For all 40 trips analyzed, the average duration of a complete trip was 3.5 ± 0.8 h, ranging from a minimum of 0.8 h to a maximum of 4.9 h [14]. The segment during road transport had an average of 2.4 ± 0.8 h, ranging from 0.9 to 4.2 h. A total of four pigs were found DOD for all trips monitored, out of approximately 7000 market-weight pigs transported.

3.1. Evaluation of Hot Weather Trips

3.1.1. Effects of Trailer Management Methods for Hot Weather

Table 2 lists descriptive statistics for the 20 transport trips categorized in thermal categories *Mild*, *Warm*, and *Very Hot*. Variables summarized include number of trips, the mean (±standard deviation) of outside temperature, trip duration, and waiting time are provided. The range in average outside temperature varied from 16.7 to 35.3 °C. Mean trip duration averaged 2.1 to 2.7 h, and average waiting times before unloading varied from 4 to 28 min.

Table 2. Descriptive statistics for 20 monitored transport trips categorized as thermal categories *Mild*, *Warm*, and *Very Hot*.

Thermal Category	Misting at Loading	Trips (N)	Total Trips (N)	Mean T_{out} (°C)	Mean Transport Duration (h)	Waiting Time before Unloading (min)		
						with Fans	with Fans and Misting	No Cooling
Mild	None	8	8	16.7 ± 4.2	2.1 ± 0.6	18 ± 36	N/A [1]	28 ± 19
Warm	None During After	1 3 3	7	27.0 ± 4.2	2.3 ± 0.5	15 ± 25	4 ± 10	17 ± 16
Very Hot	During After	2 3	5	35.3 ± 1.9	2.7 ± 0.7	9 ± 12	10 ± 16	10 ± 5

[1] N/A: there were no instances of this combination occurring.

Table 3 provides results of effects of trailer misting methods for hot weather management for the corresponding trips included in Table 2. There was no effect of zone on any of the measured variables for these hot weather trips. Results for the main effects (thermal category, trailer zones, and misting methods) are described in the following paragraphs.

Table 3. Evaluation of the effects of thermal category, trailer zones, and misting methods for 20 monitored transport trips categorized as *Mild*, *Warm*, and *Very Hot*. The response variables analyzed included average temperature difference between trailer interior and the outside temperature, average and the maximum temperature and humidity index (THI), and the difference between maximum THI and the average outside THI condition.

Response Variable Analyzed	Thermal Category					Trailer Section (Zones) [1]								Misting at Loading				
	Sig. [2]	*Mild*	*Warm*	*Very Hot*	SEM	Sig. [2]	Front		Middle		Rear		SEM	Sig. [2]	None	During	After	SEM
		(8) [3]	(7) [3]	(5) [3]			1	4	2	5	3	6			(9) [3]	(5) [3]	(6) [3]	
Average temperature difference between trailer interior and T_{out} (°C) [4]	**	1.1^{A}	0.5^{B}	-1.1^{C}	0.5	NS	0.4	0.1	0.1	−0.2	0.4	0.1	1.1	***	0.9^{A}	-1.0^{B}	0.5^{A}	0.5
Average THI [5]	***	65^{A}	75^{B}	84^{C}	0.8	NS	75	75	74	74	74	74	4.4	NS	75	75	74	1.4
Maximum THI [6]	***	73^{A}	80^{B}	87^{C}	0.9	NS	81	81	79	80	80	81	4.6	**	77^{A}	82^{B}	82^{B}	1.2
Maximum THI difference [7]	***	12.2^{A}	7.4^{B}	4.9^{C}	1.1	NS	9.2	8.6	7.2	7.4	7.6	8.6	2.2	***	4.9^{A}	8.4^{A}	11.1^{B}	1.1

[1] Trailer zones are designated as: zone 1—top front; zone 4—bottom front; zone 2—top middle; zone 5—bottom middle; zone 3—top rear; and zone 6—bottom rear. [2] Sig. indicates level of significance for the main effect, where ** indicates a *p*-value < 0.01, *** indicates a *p*-value < 0.001, and NS is not significant ($p > 0.05$). [3] Numbers in the parenthesis indicate the number of trips completed and analyzed for this combination. [4] Numbers indicate the least square means for the average temperature difference between trailer interior and the outside temperature for the during transport segment. [5] Numbers indicate the least square means for the average THI for the during transport segment. [6] Numbers indicate the least square means for the maximum THI for the during transport segment. [7] Numbers indicate the least square means for the difference between the maximum THI documented for inside the trailer and the average THI for the outside condition for the during transport segment. [A–C] Different superscripts within a row under the analyzed factor indicate the means differ significantly ($p < 0.05$) using the Tukey–Kramer test for differences of least squares means.

Thermal category. Results from Table 3 show that the thermal category had a significant effect on the trailer interior temperature rise. For *Mild* and *Warm* categories, trailer interior was warmer than T_{out} but cooler for the *Very Hot* trips, presumably from evaporative cooling of mist water applied prior to transport. When compared to the *Mild* category, average THI was higher for the *Warm* and *Very Hot* categories. THI levels between 78 and 84 are considered dangerous for livestock animals, and THI levels greater than 84 constitute an emergency condition [14,15,25]. We observed dangerous average THI conditions during transport for all trips in the *Very Hot* category, and both dangerous and emergency maximum THI for *Warm* and *Very Hot* categories. The difference between maximum THI recorded in the trailer and the average outside THI was notably different between thermal categories ($p < 0.001$). The difference of maximum THI decreases when the outside temperature became higher.

Trailer section. Trailer sections (front: Zones 1 and 4; middle: Zones 2 and 5; and rear: Zones 3 and 6) did not affect the average temperature rise, average THI, or maximum THI. The trailer thermal environment was uniformly hot during each of these trips. The uniform distribution of temperature rises during hot weather trips agree with the results reported by [10], where no difference in temperature among compartments of a double-decked pot belly trailer for weaned piglets was observed for trips with 29 °C average ambient temperature. They did not report the THI conditions in their study.

Misting. Misting before the start of trip slightly cooled the trailer for hot weather categories, as measured by average temperature rise in the trailer ($p < 0.001$), maximum THI recorded ($p < 0.01$), and the difference between maximum THI and average outside THI ($p < 0.001$). However, the variation in average temperature rise between no misting, misting during loading, and misting after loaded was less than ±1 °C. Only misting during loading resulted in an average interior temperature cooler than outside. One explanation may be that the average temperature difference over the entire transport segment may mask any relatively shorter-term cooling benefits from misting, which usually only lasted 5–15 min. This is further confirmed by average THI which was also not different between misting at loading categories ($p > 0.1$). A previous study [36] that assessed thermal environment in a goose-neck horse trailer during summer conditions reported that the THI was not uniform in the trailer, and more extreme conditions were found toward the front, suggesting that the front section openings served as outlets. The THI in their trailer was more affected by the ambient condition rather than different trailer positions [36], which matches with our results in which the maximum THI difference was found in the top front zone ($\Delta THI_{max} = 9.2$), although our study did not note any statistical difference in any THI responses for different zones. The effects of misting after loading, was a higher average temperature rise than misting during loading ($p < 0.05$). The effects of no misting were similar for mean THI to both misting conditions, and a lower maximum THI ($p < 0.01$) than either misting condition, and a lower ΔTHI_{max} ($p < 0.001$) than misting after loading. These results show that one can expect misting during loading to result in similar or reduced interior temperature, similar average THI, and higher maximum THI in the trailer during transport compared to no misting. The use of misting pushed the thermal environment to a dangerous condition for at least some portion of the transport segment, although it did not have a lasting effect.

3.1.2. Temporal Thermal Profile inside Trailer during Hot Weather

Obtaining temperature and THI profile of the trailer during the entire course of the transport trip is helpful to understand the nature and to assess the variability of the interior thermal conditions that are encountered by pigs. The spatiotemporal thermal profile was investigated for both hot and cold weather conditions. Figure 2 and Figure 3 show the change of center-zone temperatures and the THI respectively from the six trailer zones with elapsed time from the time the trailer left the home base until the trailer was completely unloaded at the abattoir. The two example trips during hot weather shown here are: (a) a morning trip in the *Very Hot* category that was completed from 5:12 a.m. to 1:24 p.m. and (b) an afternoon trip in the *Warm* category, from 9:45 a.m. to 5:20 p.m. The outside temperature is also plotted on Figure 2, and the trip segment and trailer management were numbered with explanation provided.

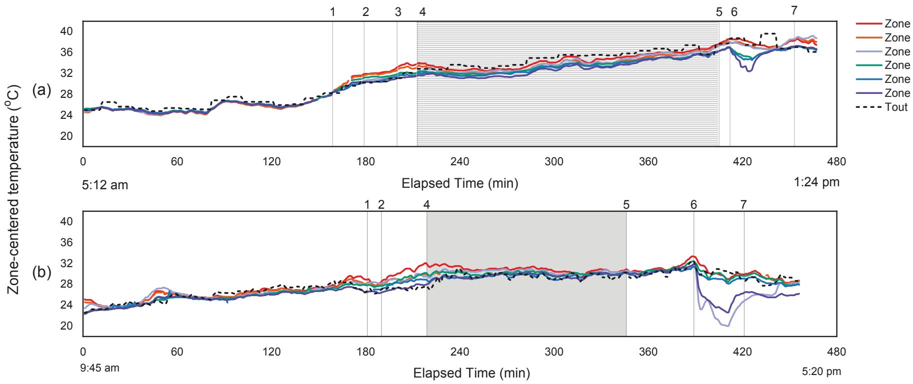

Figure 2. Ceiling-centered temperature profile representing 6 zones inside a pig trailer during: (**a**) a *Very Hot* morning trip; and (**b**) a *Warm* afternoon trip. Zones 1 to 3 represent trailer top deck, and Zones 4 to 6 represent bottom deck. Events occurred during the trip are numbered on the figure as follows (if present): **1.** Arrival at a commercial pig barn; **2.** Loading; **3.** Misting applied inside trailer; **4.** En route; **5.** Arrival at abattoir; **6.** Access to fans and misting during waiting; **7.** Unloading.

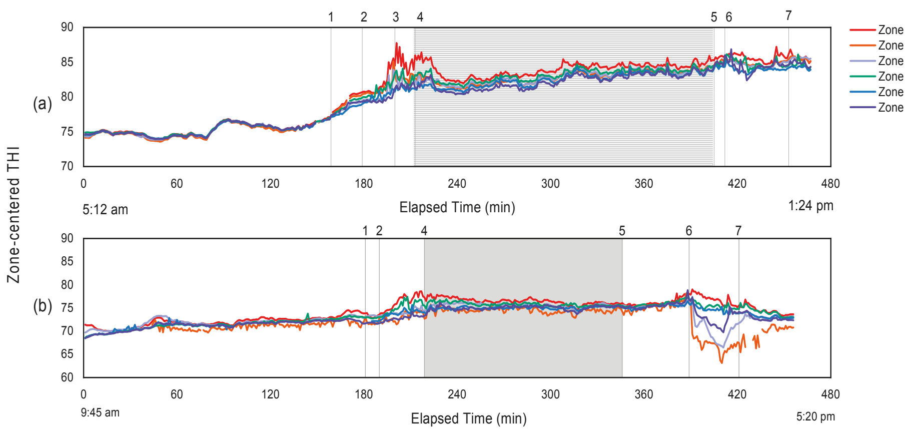

Figure 3. Ceiling-centered THI profile representing 6 zones inside a pig trailer during: (**a**) a *Very Hot* morning trip; and (**b**) a *Warm* afternoon trip. Zones 1 to 3 represent trailer top deck, and Zones 4 to 6 represent bottom deck. Events occurred during the trip are numbered on the figure as follows (if present): **1.** Arrival at a commercial pig barn; **2.** Loading; **3.** Misting applied inside trailer; **4.** En route; **5.** Arrival at abattoir; **6.** Access to fans and misting during waiting; **7.** Unloading.

Figure 2 and Figure 3a,b demonstrate how trailer interior zone temperatures and THI paralleled the outside temperature during summer conditions, except after arrival at the abattoir when misting with fans was used. In Figure 2, all six zones followed T_{out}, with some zones showing more noticeable changes after cooling was applied, and to a lesser extent, prior to the start of road transport as the trailer heated up. The six zone temperatures are nearly identical to the outside temperature when the trailer was empty driving on the road without pigs. Once the trailer stopped for loading, the zone temperatures began to diverge, and then became more uniform again during the transport segment. During loading and cooling, Zones 1 and 2 (top front and middle) were the warmest, although zone effect on average temperature rise was not significant (Table 3). The center-zone THI conditions (Figure 3) paralleled the temperature profile over time (Figure 2), except that the THI substantially increased to approximately 83–85 after misting was applied in the trailer for the trip shown in Figure 2 and Figure 3a, suggesting that the pigs experienced a temporarily dangerous thermal comfort condition for most zones, and an emergency condition for Zone 1. However, this change in thermal comfort condition was not illustrated by the temperature history profile. In the other trip that did not receive misting at loading, the THI in six zones increased when pig loading started, but to a much lesser extent than that with misting applied simultaneously. The visualization of the THI history profile supports our statistical analysis results for the ΔTHI_{max}, where an average increase in THI of 4.9 is noted for no misting applied (same case as represented by the trip in Figure 3b), and an increase of 11.1 in THI for misting after loaded (same case as represented by the trip in Figure 3a). In Figure 3a, the discomfort THI condition lasted about 30 min from the onset the misting, and approximately 10–15 mins into the transport segment, but not the entire transport duration. This supports the results that no variation in trailer zones was found for the average THI. For these two representative trips, Zones 1 and 4 (trailer front section) had the most extreme thermal conditions with pigs present for both temperature and the THI. However, this variation between zones were likely masked by the statistical analysis over the entire transport segment.

With regard to misting with fans before unloading used in both trips, substantial non-uniformity in zone temperature and THI was observed. In Figure 2a, interior trailer temperatures in Zones 4–6, representing the trailer bottom deck, rapidly decreased soon after access to misting following fans, resulting in a minimum 4 °C difference between the trailer top and bottom decks; the magnitude of difference was even greater for the trip of Figure 2b, with Zones 5 and 6 showing a temperature reduction of 10 °C cooler than the other zones. In Figure 3a, the THI in the same zones (4–6) decreased similarly to that of the temperatures, but the variation between zones was smaller than that in Figure 2a. In Figure 3b, the THI in both Zones 5 and 6 paralleled the temperature decrease. In addition, the THI in Zone 2 dropped substantially when receiving fans and misting, although the temperature profile in Zone 2 did not show such trend. This further evident that the cooling effect was not uniform in all trailer zones. One explanation for the different zone thermal responses for these two is the uncontrolled trailer parking position, providing different airflow coverage for different parts of the trailer. The uneven benefits from cooling methods were also seen by the pig surface temperature analysis from the same study [14,15]. In a study conducted on broiler transport [37] with external fans at the side of the trailer, simulated ventilation patterns and velocities using a Computational Fluid Dynamics (CFD) model were not uniform for all locations. They concluded that little of the air flow generated by the fans entered the trailer. By adjusting fan heights, they observed a 41% difference in air flow rate in two adjacent top trailer rows, which supports our observation that only the two to three zones on the bottom deck showed any positive effect of fans at the abattoir.

3.1.3. Spatiotemporal Variability inside the Trailer during Hot Weather

While the temporal thermal history plots in Figure 2 and Figure 3 illustrate variations in zone temperature and THI over time, the animations of spatial variability in the top and bottom sections of the trailer provide a more detailed view of the thermal variation within and between zones. Figure 4a–c provide animation screenshots for three procedures (misting during loading, as transport is started, and waiting by fans after misting was applied at the abattoir) during a representative *Very Hot* monitoring trip (average T_{out} > 32 °C). The first two procedures, misting during loading and at the start of a trip show substantial spatial variability in both bottom and top decks associated with the misting coverage. During misting, the center zones in the bottom deck were as much as 8 °C cooler than the middle and rear of the top deck. Analysis using only the average temperature of the thermistors, such as depicted in Figure 2, will likely mask this large temperature variation. About 15 min after misting was stopped, the trailer was about to embark on a 2 h journey (Figure 4b), and there were still residual cooling effects noted especially on the lower deck. Upon arrival at the abattoir, misting was applied, and the truck was then moved to the fan bank. The resulting upper-deck temperature is uniform, but extreme (36–38 °C) as was most of the lower deck except in the very center section where some evaporative cooling was still taking place.

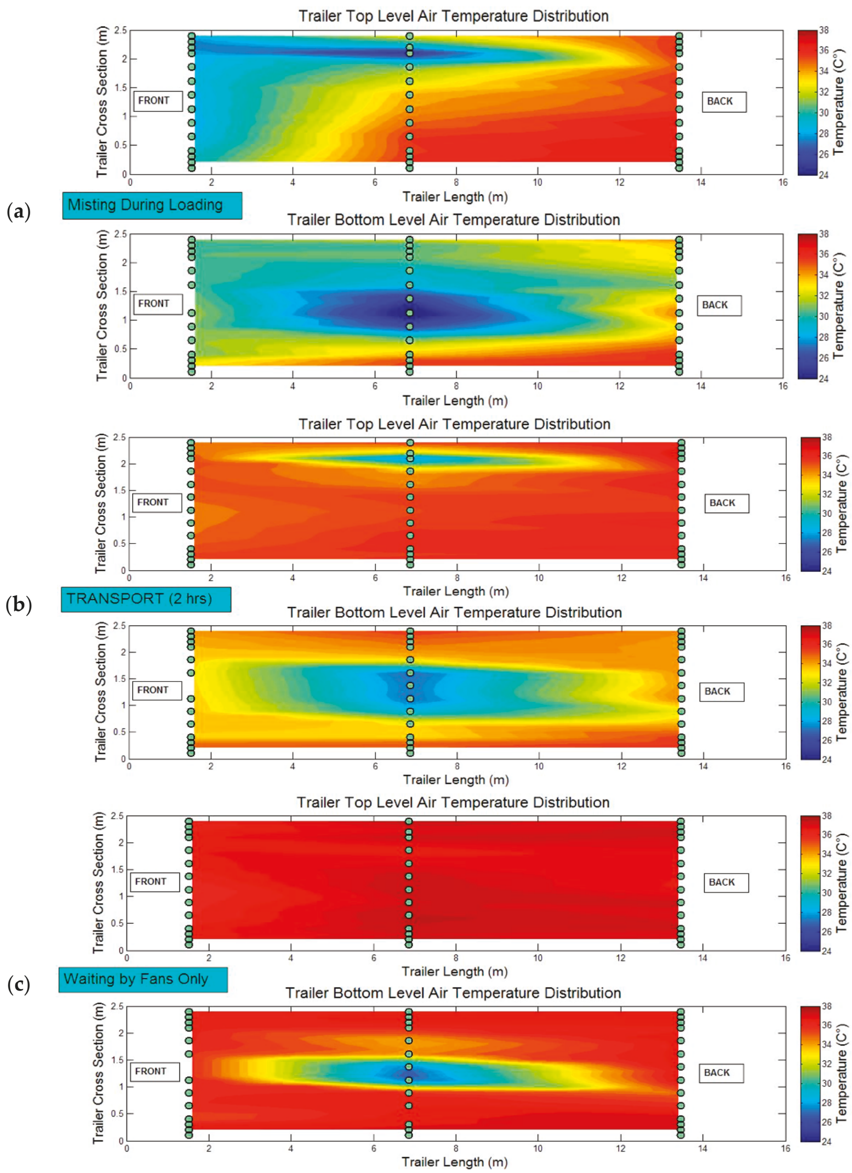

Figure 4. Trailer interior temperature distribution for three events during a hot summer monitoring trip (T_{out} > 32 °C). Lighter color areas represent cooler temperatures inside the trailer, and potentially the cooling effects of the misting lasted into the transport segment. The three events were: (**a**) trailer was stationary, and misting was applied inside onto the pigs and bedding; (**b**) trailer was moving; and (**c**) stationary trailer loaded with pigs at abattoir waiting besides external fans with misting previously applied. The green circles indicate position of air temperature sensors at the pig level.

These screenshots, and the complete animations provided in the Supplemental Material to this article, illustrate the lack of uniformity in spatial temperature. In the example in Figure 4, this is likely from unequally distributed misting nozzles across the trailer, and the limited effectiveness of the fans at the abattoir.

Misting and fan operation for cooling during hot weather created large temperature variation within the trailer and was not applied uniformly to all pigs, which can be observed in the sample data visualizations (Figure 2, Figure 3 and Figure 4). Misting at loading showed cooling benefits that lasted into the road transport segment, but increased values of THI for at least some portion of the trip. Similar cooling effects that lasted into the road transport segment were observed for misting either during loading or after loaded, but no conclusion can be derived regarding which misting method results in greater temperature depression. The efficacy of misting is affected by the location and direction of the misting nozzles such that the coverage area is optimized, and critically, requires high and uniform velocity distribution to ensure evaporation during transport.

3.2. Evaluation of Cold Weather Trips

3.2.1. Effects of Trailer Management Methods for Cold Weather

Results of the effects of trailer boarding and bedding on average temperature difference for *Cold* and *Very Cold* thermal categories is shown in Table 4. Statistical significance between the means for different levels of the analyzed factors is indicated by superscripts in the same row. Boarding position x trailer zone interaction was not significant, thus not discussed.

Trailer Section. The trailer zones have a significant effect on the average temperature rise between trailer interior and the outside, indicating the thermal environments between trailer zones were significantly different during transport ($p < 0.001$). For the 16 trips in the *Cold* category, the front section of the trailer (Zones 1 and 4) was warmer than the middle and rear sections, while the middle section (Zones 2 and 5) and rear section (Zones 3 and 6) were not different from one another. For the *Cold* category, the front section was the warmest area of the trailer, while the rear section was the coolest, which indicates the trailer rear section was the air inlet and the front section as the outlet. This result is not consistently the same as that of the hot weather analysis, which reveals that different trailer sections responded to the environment differently between cold weather and hot weather conditions.

Boarding—Bedding Combination and Boarding Position. Although Table 4 shows that the main effect of the boarding and bedding combination was significant for the average temperature rise between trailer interior and T_{out}, heavier boarding and bedding combination did not show any benefits for increasing trailer zone temperatures. Similarly, for the 16 trips analyzed, none of the boarding positions (as a nesting factor in the boarding—bedding combination) yielded warmer trailer interior temperatures than another. Furthermore, the average temperature rise between trailer interior and the outside was not different based on boarding distribution. More boarding could reduce the air exchange rate by limiting the air inlets and exhaust areas and, hence, the air circulation patterns as well. Since air circulation patterns are affected, air velocity over the animals can also be affected, which in turn theoretically could change convective heat loss. However, our earlier analysis on pig surface temperature as a function of 25% vs. 50% boarding showed no significant difference [15]. Our results agree with [30–32], who reported no difference between trailer boarding levels for pig mortality or morbidity for temperature ranging from 5.1 to 23.3 °C; and bedding level did not affect the mortality or morbidity rate at the abattoir.

Table 4. Evaluation of the effects of trailer zones, bedding and boarding combination, and boarding position for 20 monitored transport trips categorized as thermal categories *Cold* and *Very Cold*.

Thermal Category	Trips (N)	Mean T_{out} (°C)	Mean Duration (h)	Average Temperature Difference between Trailer Interior and T_{out} (°C) [1]																			
				Trailer Section (Zones) [2]								Boarding—Bedding					Boarding Position						
					Front		Middle		Rear				LM	MM	MH			LM		MM	MH		
				Sig. [3]	1	4	2	5	3	6	SEM	Sig. [3]	(6) [4]	(5) [4]	(5) [4]	SEM	Sig. [3]	Even (3) [4]	Rear (3) [4]	Even (5) [4]	Rear (2) [4]	Back (3) [4]	SEM
Cold	16	7.0 ± 2.4	2.5 ± 0.8	***	3.9^{AB}	5.0^{A}	2.1^{C}	2.3^{BC}	1.8^{C}	2.1^{C}	0.9	**	3.6^{A}	2.6^{B}	2.5^{B}	0.6	NS	4.0	3.2	2.6	2.3	2.6	0.6
Very Cold	4	−10.6 ± 3.4	2.3 ± 1.0	**	13.6^{A}	13.6^{A}	8.1^{BC}	14.4^{A}	4.5^{C}	11.4^{AB}	3.4		NA [5]					NA [5]					

[1] Numbers are the least square means for *Cold* category and means for the *Very Cold* category. [2] Trailer zones are designated as: zone 1—top front; zone 4—bottom front; zone 2—top middle; zone 5—bottom middle; zone 3—top rear; and zone 6—bottom rear. [3] Sig. indicates level of significance for the main effect, where ** indicates a p-value < 0.01, *** indicates a p-value < 0.001, and NS is not significant ($p > 0.05$). [4] Numbers in the parentheses indicate the number of monitoring trips completed and analyzed for this combination. [5] NA indicates insufficient observations across levels of main effect for testing. $^{A–C}$ Different superscripts within a row under the analyzed factor indicate the means differ significantly ($p < 0.05$) from the Tukey–Kramer test for differences of least squares means.

3.2.2. Temporal Thermal Profile inside Trailer during Cold Weather

Figure 5 show the change of center-zone temperatures from the six trailer zones with elapsed time for a complete transport trip in the *Very Cold* category that was conducted during the evening, from 6:00 p.m. to 1:45 a.m. the next day.

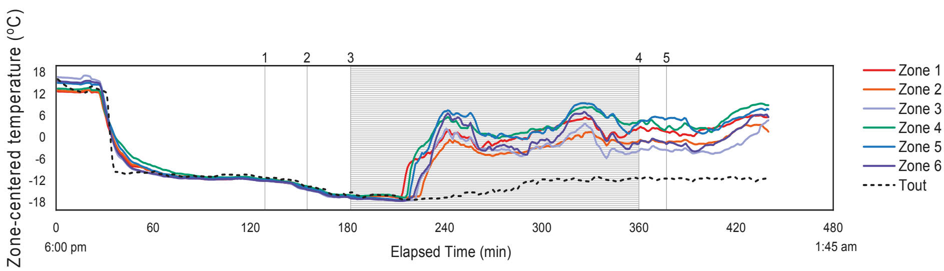

Figure 5. Ceiling-centered temperature profile representing 6 zones inside a pig trailer during a *Very Cold* evening trip. Zones 1 to 3 representing trailer top deck, and Zones 4 to 6 representing bottom deck. Events occurred during the trip are numbered on the figure as follows: **1.** Arrival at a commercial pig barn; **2.** Loading; **3.** En route; **4.** Arrival at abattoir; **5.** Unloading.

For this winter monitoring trip, variations of up to 8 °C between zone temperatures were observed. The temperature rise added by the pigs' heat production was more obvious than that of the summer trips, reaching an 18 °C temperature difference on average between trailer interior and T_{out} which was about −14 to −12 °C for most of the trip. This trend was similar to that found by [10,11], where a magnitude of 15–20 °C temperature rise in the trailer was noted for winter trips conducted for weaned piglets transport in Illinois and Iowa. Trailer temperatures during the initial part of the road trip were similar to outside, until about 40 min into the trip when the trailer began to warm up. Zones 3 and 6 (rear of trailer) were consistently colder after the end of the trip, but during transport no clear trends in differences between trailer zones or trailer decks can be seen in this plot, although the front was warmer on average for all cold trips ($p < 0.05$). However, the maximum difference between zone temperatures was 12 to 15 °C. Reasons for this large variability are not clear, but it is likely the amount and placement of boarding affected the relative position of inlets and outlets for the trailer.

3.3. Ventilation Implications

Our analysis for cold weather conditions, based on variation in zone temperature rise, showed that the trailer front section was consistently the warmest, while no difference was noted for other zones. For warmer weather trips, no significant difference in the trailer interior temperature rise, average and maximum THI, or maximum THI difference was found between different trailer zones, which indicates a uniform spatial thermal environment. Despite the lack of statistical significance in average temperature rise and THI, our visualized sample data sets observed the most extreme conditions in the front zone for a *Very Hot* and a *Warm* trip. Our results are substantially in agreement with [10], where thermal environment and ventilation patterns were studied for a double-decked pot belly trailer transporting weaned piglets. They reported that the top front section was consistently the warmest location on the trailer for multiple cold weather trips (average ambient temperature of 2 °C, equivalent to the *Cold* category in this study), while the front–middle compartments on both decks, and the top rear compartment, were found to be the warmest locations for trips with average ambient temperature 16 °C (equivalent to the *Mild* category in this study). The middle compartments of both decks were coolest for both ambient temperatures studied. However, no difference in compartment temperature was noted for trips with a higher average ambient temperature (29 °C) which fits the *Warm* category in this study.

Variability of the thermal environment in the trailer can be a useful tool for indication of potential ventilation patterns in a moving trailer. In colder conditions, cooler zone temperatures indicate where air is entering the trailer, and warmer temperatures indicate locations with air outlets and likely less air velocity [10,11,16,38,39]. Previous livestock trailer ventilation studies demonstrated that air inlets are typically at the rear and air outlets toward the front [3,16,38–41]. Our results agree with this situation for colder weather conditions; the front of the trailer was consistently the warmest area while cooler temperatures were recorded toward the middle to rear sections, suggesting a general air flow from rear to front. During winter trips, the front nose vents were completely covered in this study, much of the side openings were boarded, and only a portion of the rear section could serve as an air inlet. The cold air heats while moving inside the trailer due to sensible energy contributions from the pigs. A greater temperature increase in one area can indicate either less ventilation in that area, or a sufficiently low ventilation rate to allow for sensible heating. In this study, our results indicate a ventilation "dead-spot" at the front during winter, suggesting a relatively low overall ventilation exchange rate, and which resulted in non-uniform thermal distribution. These non-uniformly distributed ventilation patterns are acknowledged by research with multiple livestock animals [10,11,16,37–39]: Purswell et al. [16] reported the air exchange rates were notably different across different locations on a slant-load horse trailer, and varied with road speed; Harmon et al. and Zhao et al. [10,11] simulated air flow rate using a 1/7th scale trailer model, and reported a range of 3.4 to 6.9 ms^{-1} air velocity can be expected in a double-decked pot-belly pig trailer; Heymsfield et al. [37] reported different ventilation patterns and rates across cages in a straight-deck broiler transport trailer.

For warm-weather trips, the trailer temperature distribution was relatively uniform in this study, suggesting a different ventilation pattern or rate, as side and front openings are opened completely and the ventilation rate becomes sufficiently high to minimize temperature variation. The trailer nose vents located at the front corners were completely closed during winter but opened during summer (as can be seen from Figure 1, in which the four nose vents are all open; the left corner of trailer front board had two identical vent openings as shown on the picture). During hot weather, it is likely that the fresh air entered into the rear sections of trailer and exited towards the front. With trailer sides completely open and front nose vents uncovered, the lack of temperature or THI rise was the result of sufficient opening areas and the pressure gradient to induce a high ventilation rate, limiting the temperature rise observed during winter.

The varying ventilation patterns or rates can be attributed to many factors: during different seasons, trailer speed, vent openings, and the presence of the rear door panel significantly affected the air exchange rates in a horse trailer [16,38]; front trailer vents notably affected the direction but not the speed of air flows [10]; and interior pen partitions reduced air velocity (hence, air flow) by about 50% [10,11]. Given the relatively solid floors on each deck, the top and bottom decks are effectively independent of each other, with airflow through them dictated by size and location of potential inlets and outlets. A few previous studies supported the possibility that different configurations of side opening covering and rear panel design may also affect the ventilation pattern in the trailer [10,15,16,38]. Our results show that heavier boarding did not appear to reduce ventilation rate, as indicated by a greater average temperature difference for the lightest boarding scenario (Table 4), but the position of boarding likely altered the location of air inlets and exhausts along the sides of the trailer. When placing boarding equally versus distributing it more toward the rear during cold weather, no potential for increasing trailer temperature was observed. These explanations cannot be directly verified because air flow direction and velocity inside the trailer were not directly measured in this study.

4. Conclusions

Variation in the six zones within a commercial transport trailer over the course of a year were tested for lack of uniformity against different factors depending on outdoor weather conditions. For 20 trips conducted during *Mild*, *Warm* and *Very Hot* conditions, no significant variation was observed in temperature rise or average THI between the six trailer zones. Ventilation through the trailer was sufficient to limit temperature rise due to sensible and latent heat contributions by the pigs. Misting during loading the trailer with pigs showed modest benefit to cool the trailer interior temperature to less than outside temperature, however, misting did not improve the average THI and created short term heat stress conditions as measured by maximum THI in the trailer, pushing the thermal environment to a potentially dangerous condition for at least some portion of the transport segment. After transport, fans with or without misting reduced temperature rise in some zones of the trailer but the benefits were not uniform.

For 20 *Cold* and *Very Cold* weather trips, the trailer interior thermal environment was not uniform, with the front top and bottom zones being the warmest, indicating less ventilation in these areas, which is in line with results from animal transport assessments for pigs and other species. Boarding/bedding combinations changed ventilation rates but was generally inconsistent with respect to variation in temperature, and boarding position had no measurable effect on ventilation pattern or rate. Unlike the warmer weather situation with full ventilation, a partially boarded trailer reduced the ventilation rate and resulted in a temperature gradient from the rear towards the front, conforming to the air inlet and outlet locations, respectively. The potential implications of reduced ventilation would be a susceptibility to poorer air quality and potentially challenging thermal environment for the pigs, which have significant consequences for pig welfare during cold weather transport.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-2615/8/11/203/s1, Animation A1: Animation of a complete commercial pig transport trip during very hot weather condition (1). Animation A2: Animation of a complete commercial pig transport trip during very hot weather condition (2). Animation A3: Animation of a complete commercial pig transport trip during warm weather condition. Animation A4: Animation of a complete commercial pig transport trip during mild weather condition. Animation A5: Animation of a complete commercial pig transport trip during very cold weather condition.

Author Contributions: Conceptualization, Y.X., R.S.G. and A.R.G.-M.; Methodology, A.R.G.-M., R.S.G. and Y.X.; Software, Y.X.; Validation, Y.X.; Data Analysis, Y.X., R.S.G. and A.R.G.-M.; Writing-Original Draft Preparation, Y.X. and R.S.G.; Writing-Review and Editing, Y.X., R.S.G. and A.R.G.-M.; Visualization, Y.X.; Supervision, A.R.G.-M.; Project Administration, A.R.G.-M.; Funding Acquisition, A.R.G.-M.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix

The descriptive information of the animations is interpolated from pig-level air temperature sensors for five representative trips that covered extreme outside thermal categories and encountered representative combinations of critical trailer management. The contents were discussed in Materials and Methods, Results, and Discussion. Complete animations can be accessed from the Supplementary Materials.

Table A1. Descriptive information of the five animations with reference to pages 6, 11–13.

	Animation 1	Animation 2	Animation 3	Animation 4	Animation 5
Outside Temperature	>32 °C	>32 °C	27–32 °C	10–20 °C	<−12 °C
Thermal Category	*Very Hot*	*Very Hot*	*Warm*	*Mild*	*Very Cold*
Trailer Management	Misting	Misting	None	None	90% boarding
Cooling at the Abattoir	Fans & misting	None	Fans & misting	None	None
Transport Duration (h)	3.2	2.0	2.1	1.0	2.5

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Apulo-Calabrese and Crossbreed Pigs Show Different Physiological Response and Meat Quality Traits after Short Distance Transport

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Simple Summary: Transport is a stressful procedure that can affect adversely the welfare of pigs and pork quality. It is well known that response to the journey is influenced by the genetic type. However, very little is known on the response of local breeds to the transport procedures despite the increasing interest in the welfare of animals during transport. The objective of this study was to investigate the effect of short distance transport on behaviour, blood parameters and meat quality traits of Apulo-Calabrese (local Italian pig breed) and crossbreed [Duroc × (Landrace × Large White)] pigs. Statistical analyses were done using univariate and multivariate approaches. Both approaches showed that glucose, albumin/globulin, urea, and aspartate aminotransferase concentrations were influenced by the genetic type. Despite at loading Apulo-Calabrese pigs were less reluctant to move and showed a lower vocalization, physiological response to the short distance transport was more intense in this breed when compared with crossbreeds. With regards to meat quality, higher a* and lower L* coordinates were found in Apulo-Calabrese which indicates darker and more reddish meat than crossbreeds. The results from this study may provide insight into the response of local breeds to the transport procedures.

Abstract: Despite the increasing interest in the welfare of animals during transport, very little is known on the response of local pig breeds to the transport procedures. This study aims to compare the effect of short journey on behaviour, blood parameters, and meat quality traits in 51 Apulo-Calabrese and 52 crossbreed [Duroc × (Landrace × Large White)] pigs. All the animals were blood sampled five days before delivery (basal condition) and at exsanguination for the analysis of creatine kinase, cortisol, glucose, lactate, albumin, albumin/globulin, total protein, urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase, alkaline phosphate, sodium, and potassium. Post mortem pH, color, drip loss, cooking loss, and Warner-Bratzler shear force were measured at different times in *longissimus thoracis* samples. Univariate and multivariate analyses showed that glucose, albumin/globulin, urea, and AST at exsanguination were influenced by the genetic type. Apulo-Calabrese showed the highest increase in blood values of lactate, creatinine, sodium and potassium after the short distance transport. Behavioural occurrences were similar in both genetic types during unloading and lairage. Small differences were observed for meat quality although significantly higher a* and lower L* were found in Apulo-Calabrese pigs, showing meat with a deeper red colour than crossbreeds.

Keywords: pigs; local breed; Apulo-Calabrese; transport; short distance; blood parameters; meat quality

1. Introduction

Stress associated with transport has been documented in pigs by a large number of studies [1–3]. According to literature, transport stress can cause changes in the behaviour and normal physiological function affecting negatively the welfare of the animals and meat quality attributes [4,5]. It is well known that response to the journey can be influenced by the genotype [6], and factors such as temperature and humidity [7,8], truck conditions [9,10], transport, and/or lairage durations [11] and handling of the animals [12]. Blood parameters at exsanguination have largely been used to assess the stress of transport in livestock [13–15].

In pigs, extensive studies on blood parameters after transport have been carried out in conventional commercial breeds and their crossbreeds, whilst little information exists on local pig breeds such as Erhualian [16] and Basque [17].

Among the indigenous Italian pig breeds is Apulo-Calabrese which is included in the list of endangered breeds by the United Nations' Food and Agriculture Organization [18] and registered in the herd book held by the Italian National Association of Pig Breeders (Associazione Nazionale Allevatori Suini, ANAS). In the year 2017, the breeding population counted 540 sows and 63 boars reared in 63 farms, 31 of which can be found in the Calabria region [19] Apulo-Calabrese is a black-skinned, medium-sized pig with small socks on the forelimbs and large socks on the hind limbs [20]. It is well adaptable to different production systems and can be reared outdoor or indoor in a conventional system [21]. Meat from Apulo-Calabrese is processed into four Protected Designation of Origin (PDO) salami, typical of the Calabrian region [20]. In Italy there is an increasing interest in the welfare of local breeds due to the growing consumer preference for PDO animal friendly products, however, existing research does not provide information on the response of this breed to the transport procedures.

The aim of this study was therefore to investigate the effect of short distance transportation on behavioural response, blood parameters, and meat quality traits of Apulo-Calabrese with respect to crossbreeds.

2. Materials and Methods

2.1. Animals

Blood collection at the farm was carried out by a veterinarian in conjunction with routine sampling for sanitary controls. Transportation and slaughter of all pigs were carried out in compliance with EC regulation No 1/2005 and EC regulation No 1099/2013, respectively.

Fifty-one Apulo-Calabrese pigs registered in the herd book of ANAS and 52 crossbreeds [Duroc × (Landrace × Large White)] were used. Apulo-Calabrese pigs were born in this farm from the mating of 13 sows by seven boars of Apulo-Calabrese whilst crossbreeds were bought at about 30 kg live weight from another piggery in the same region. All the pigs were fattened in the same finish facility in separate pens (7–10 pigs per pen) according to their genetic type and were fed the same commercial diet (14,644 KJ DE/kg, 155 g crude protein/kg, 22 g crude fat/kg, 80 g lysine/kg, 58 g ash/kg) in a liquid feeding system with dry feed and water mixed in a 1:4 ratio. All the pigs were identified by a numbered plastic ear tags. Apulo-Calabrese pigs were slaughtered when they reached 135 kg live weight (364 ± 58 days old) due to their slow growth whilst crossbreeds were slaughtered at approximately 155 kg live weight (300–330 days old).

2.2. Pre-Slaughter and Slaughter

Approximately 12 h prior to transport, feed was withdrawn. Loading was carried out at 7 a.m. using a mobile ramp (length 4.5 m, width 0.7 m, with solid side walls of 1.0 m and adjustable height) available at the farm. Pigs were delivered through three consignments to the slaughterhouse. At each delivery, pigs from the four pens were herded by electric prods and walked the same distance (25 m) to reach the ramp which was positioned in correspondence with the facility door. The lorry was a hydraulic three tier equipped with internal partition and mechanical ventilation on the left side.

Pigs were transported for approximately 1 h to a local processing plant (Piano Lago, Cosenza, Italy) on two decks with a space allowance of about 0.50 m^2/100 kg live weight. Unloading was done using the ramp of the lorry and pigs were driven for 10 m to the lairage pens where they rested for 30 min. Outdoor temperature and relative humidity were recorded for each journey by a thermo-hygrometer (mod. HI9065, Hanna, Padua, Italy) during loading and unloading at the entrance of the ramp and at the entrance of the resting pen, respectively (Table 1). During transport and lairage, mixing between pens was avoided. The pigs were stunned by electrical tongs (head only; 220 V, 1.3 A). After stunning, exsanguination blood was collected from each pig (Table 1).

Table 1. Outdoor temperature (Temp), relative humidity (RH), and durations of loading, transport and unloading recorded in pigs in the three deliveries.

Delivery	Genetic Type	Number of Pigs	Loading			Transport	Unloading		
			Duration [1] (min)	Temp (°C)	RH (%)	Duration (min)	Duration [2] (min)	Temp (°C)	RH (%)
1	Apulo-Calabrese	19	8	10.3	79	63	2	11.2	88.4
	Crossbreed	20	16				9		
2	Apulo-Calabrese	17	10	18	72.3	67	7	19.5	65.8
	Crossbreed	17	16				3		
3	Apulo-Calabrese	15	6	21.5	59.6	60	5	19.7	65.5
	Crossbreed	15	6				2		

[1] From the opening of the farm gate until the last pig entered the lorry. [2] From the opening of the gate of the lorry until the last pig entered the lairage pen.

2.3. Behavioural Response

The behaviour recordings during loading at the farm and unloading at the abattoir included slipping, falling, reluctance to move, turning back, overlapping, and vocalization as previously described [22,23]. Lying, sitting, and standing behaviours after 25 min of resting time was directly observed for a period of 2 min.

2.4. Blood Sampling and Analysis

The animals were blood sampled five days before delivery as reference for basal blood parameter level (T0) and at exsanguination (T1) for the analysis of creatine kinase (CK), cortisol, glucose, lactate, albumin, albumin/globulin ratio, total protein, urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphate (ALP), sodium (Na^-), and potassium (K^+). All biochemical parameters except for glucose, were measured in serum obtained from blood collected in serum separator tubes with gel separator and clot activator (Vacutest Kima, Padua, Italy), let to clot and centrifuged at 1300× g at 20 °C (Eppendorf 5702R, Eppendorf, Milan, Italy) for 10 min. Plasma for the measurement of glucose was harvested from anticoagulated blood collected in Na_2EDTA test tubes containing the glycolysis inhibitor potassium fluoride (Vacutest Kima, Padua, Italy), centrifuged at 1300× g at 20 °C for 10 min. Parameters were measured by colorimetric assays on automated analyser (Olympus AU 400, Beckman Coulter, Milan, Italy) with the exception of cortisol. Serum cortisol was determined using the Kit Immulite One Cortisol (medical system code LKC01, Siemens Health Care Diagnostic Limited, Glyn Rhonwy, Gwynedd, UK).

2.5. Skin Bruises Measurement

Carcasses were horizontally exsanguinated for 5 min and then hung for 10 min before being submerged in a scalding tank for dehairing at 62 °C for 8 min. After dehairing, skin damages were subjectively assessed by a trained observer, using a four-point scale (1 = none to 4 = severe) based on the scale developed by the Danish Meat Research Institute (DMRI) [24]. The DMRI scale was used to score all skin lesions on the front (head included), middle and hind quarters of each carcass. Moreover, a skin damage score for the whole carcass was calculated using the highest score assigned to each quarter [24]. At about 40 min post mortem, carcasses were split, weighed, and transferred to the chilling room.

2.6. Meat Quality Measurements

Measurements of pH on the *longissimus thoracis* muscle (LT) at the level of six/seven thoracic vertebra were recorded at 1, 3, and 6 h post mortem on the left side, with a pH-meter (mod. HI8424, Hanna, Padua, Italy) equipped with Crison electrode (Crison Instruments, SA, Barcelona, Spain) and an automatic temperature compensation probe. At 6 h post mortem the left side was sectioned in the primal cuts and a sample of LT muscle between 6 and 10 thoracic vertebra (10 cm thickness) was collected. After 30 min of blooming, L*, a*, and b* coordinates were determined using Minolta chroma-meter (CR-300, Minolta Camera Co., Ltd., Osaka, Japan) with D65 light source and 0 °C viewing geometry. Samples were transferred (by air) to a laboratory of the Department of Agricultural and Food Sciences (Bologna, Italy). Measurements of pH were repeated at 24 and 72 h post mortem and colour measurements were repeated at 24, 72, and 144 h after slaughter. At the same time interval, cooking loss was determined on a slice of the LT muscle according to Honikel [25] and Warner-Bratzler shear force (WBSF) was measured after cooking using Instron apparatus (mod. 1140, Instron, Norwood, MA, USA). Drip loss was determined at 24 h post mortem [25].

2.7. Genotyping

In order to determine the genotype of the Halothane (ryanodine receptor 1, *RYR1*) and Rendement Napole (protein kinase AMP-activated non-catalytic subunit gamma 3, *PRKAG3*), major genes that influence the reactivity of pigs to stress and pork quality, genomic DNA was isolated from blood collected in tubes containing EDTA as anticoagulant. Genotyping of the c.599G > A single nucleotide polymorphism (SNP) *PRKAG3* gene and the g.1843C > T SNP of the *RYR1* gene were done by PCR-RFLP analyses [26,27].

2.8. Statistical Analysis

Loading and unloading duration of the two genetic types were compared using T-test. The incidence of pigs showing the different behaviours at loading, unloading and lairage were calculated and the data were processed by Fisher Exact Test procedure of SAS v. 9.3 (SAS Institute, Cary, NC, USA).

Data from blood parameters were transformed to meet assumptions of homogeneity of variance and normality of residuals. Concentrations of CK, lactate, albumin, albumin/globulin ratio, creatinine, and AST were log10 transformed. A square root transformation was used to normalize cortisol and ALT results, while an inverse transformation was used to normalize glucose and K^+ results. All transformed estimates were back-transformed for presentation to their original scale. Blood parameters were analysed using the mixed model (PROC MIXED of SAS) including the genetic type (two levels), sampling time (two levels: T0 and T1) and their interaction as fixed effects and subject within the day of slaughter as random effect. Sex as a fixed effect and hot carcass weight as a covariate were initially included in the model but they never reached statistical significance ($p > 0.05$) and were removed. Differences between means were tested by the Tukey-Kramer test ($p < 0.05$).

Data of pH, colour, cooking loss and shear force were analysed using PROC MIXED of SAS for repeated measures. The same model for blood parameters was used replacing sampling time factor with measuring time (five levels for pH, four levels for colour, three levels for cooking loss, and shear force). Sex and hot carcass weight did not reach the significant level ($p > 0.05$) and were removed from the model. The data of drip loss was analysed using the same model without measuring time.

In order to highlight possible differences between the two genetic types in blood parameters responses to short distance transport, the variation between blood parameters at exsanguination (T1) and basal blood parameters (T0) has been used to perform an unsupervised multivariate principal component analysis (PCA). All the new variables resulting from the difference between T1 and T0 blood parameters were normally distributed except for cortisol difference, which was root squared transformed in order to meet normal distribution criteria. Furthermore, a PCA has also been used to test the presence of differences in meat quality traits between the two studied genetic types. Unsupervised PCAs have been performed using *ropls* package in the R environment version 3.4.4 [28]. The data were mean centred and unit- variance scaled. The results of multivariate models were plotted on both scores and loadings plot. The combined use of univariate and multivariate analyses was employed in order to test if the results obtained with the multivariate analysis (PCA) were in agreement with what could be observed with the mixed model.

PROC GLIMMIX was used to analyse the effects of genetic type on skin damage scores recorded on each quarter separately as well as on the whole carcass. Because these data approximated a Poisson distribution, the GLIMMIX procedure's POISSON option was used. The differences in least squares means (L.S.M.) were evaluated using Tukey–Kramer's test.

3. Results and Discussion

Both genetic types did not carry the recessive allele (c.1843T) of the *RYR1* gene [27] and the dominant allele (c.599A or p.200Q) of the *PRKAG3* gene [26] that influence performance and meat quality traits [29,30].

Few research studies have been focused on the effect of transport on local breeds [16,17] although a great deal of literature exists on conventional commercial pigs and their crossbreeds [4,31,32]. The present study reports for the first time the effects of short distance transport on blood parameters and meat quality traits of Apulo-Calabrese. The results obtained showed different physiological response and meat quality attributes in both genetic types after the transport procedure.

3.1. Behavioural Recordings and Carcass Bruises

Behavioural occurrences on both genetic types were collected at loading, unloading and lairage. The two genetic types showed no differences in the behavioural occurrences during unloading and lairage, while at loading Apulo-Calabrese pigs showed significantly lower percentages ($p < 0.05$) of reluctance to move and vocalization (Table S1). During lairage, the posture was recorded after 25 min close to the end of the resting time (30 min) as planned routinely by the abattoir. For the duration of time spent in lairage no pigs were observed sitting, 94% of the pigs were lying down and only 6% of the pigs were observed standing. The genetic type did not show significant effect ($p > 0.05$) on skin damage score (whole carcass: 1.14 ± 0.34 and 1.12 ± 0.32 for Apulo-Calabrese and crossbreeds, respectively).

3.2. Blood Parameters

Table 2 shows the effects of sampling time, genetic type and their interaction on blood parameters of Apulo-Calabrese and crossbreeds.

Sampling time statistically influenced ($p < 0.05$) all blood parameters, except ALP and sodium whilst the effect of genetic type was significant ($p < 0.05$) for glucose, albumin/globulin ratio, urea, creatinine, AST and potassium. Of particular interest was the interaction between the genetic type and the sampling time since it lays emphasis on the possibility that variation of plasma components between basal and exsanguination is influenced by the genetic type. This interaction was statistically significant ($p < 0.05$) for lactate, albumin/globulin, urea, creatinine, AST, ALT, ALP, sodium, and potassium.

At exsanguination, significantly higher levels of lactate ($p < 0.05$) were found in both genetic types. Higher levels of lactate in the blood have been associated with physical stress [33]. The highest value of lactate in this study was found in Apulo-Calabrese which showed a lower concentration of basal lactate and were driven with less difficulty (minor duration) during loading, as shown in Table 1. According to Broom et al. [34], different breeds cope differently to the handling and transport procedures which could explain the higher levels of lactate found in Apulo-Calabrese in this experiment. Other welfare indicators of stress such as CK and cortisol did not differ between the two genetic types, which is in agreement with the results found by Lebret et al. [17] in the French local Basque and Large White pigs. The similar levels of basal cortisol found in Apulo-Calabrese and crossbreeds in this experiment contrasted with the result by Li et al. [16] who found higher levels of plasma cortisol in Erhualian with respect to Pietrain. Plasma glucose did not differ between both genetic types at exsanguination. However, slightly lower levels of glucose were found in Apulo-Calabrese compared with crossbreeds.

Significantly lower levels ($p < 0.05$) of albumin/globulin were found at exsanguination in Apulo-Calabrese than the crossbreeds, the values obtained were however within the normal physiological range for pigs [35]. With the exception of some globulins, plasma proteins are produced in the liver and are indicators of colloid osmotic pressure of the blood. Lower values of the concentrations may be due to a lack of dietary protein or hepatic damage [35], whilst higher values have been associated with dehydration due to the length of the journey [4].

Higher levels of urea and creatinine in the blood have been associated with food deprivation stress and an increase in physical activity as a result of the transport procedures [36]. In this experiment, significantly lower levels of creatinine ($p < 0.05$) were found in Apulo Calabrese at the basal condition when compared with the crossbreeds whereas serum urea did not differ between the two genetic types. At exsanguination Apulo-Calabrese showed significantly higher levels of urea and lower levels of creatinine when compared with crossbreeds. The values of urea and creatinine obtained in this study were within the normal physiological range for pigs and were in agreement with the results found by Dikic et al. [37] in local Turopolje breed and their crossbreeds [Turopolje × (CHypor × Swedish Landrace)].

Table 2. Effects of sampling time (T), genetic type (GT), and their interaction (T × GT) on least square means (L.S.M) and standard error of means (S.E.M.) of blood parameters of Apulo-Calabrese and crossbreed pigs on farm (baseline) and at exsanguination.

Blood Parameters	Baseline		Exsanguination					
	Apulo-Calabrese	Crossbreed	Apulo-Calabrese	Crossbreed		*p*-Values		
	L.S.M				S.E.M	T	GT	T × GT
Creatine Kinase, CK (U/L)	954.99	831.76	2089.30	2187.76	0.05	<0.0001	0.7529	0.2130
Cortisol (mg/dL)	16.72	17.38	56.31	56.73	0.26	<0.0001	0.8730	0.8690
Glucose (mg/dL)	71.23	78.13	100.57	114.59	0.00	<0.0001	0.0004	0.9497
Lactate (mg/dL)	24.23 ^{c}	33.85 ^{b}	181.01 ^{a}	143.81 ^{a}	0.03	<0.0001	0.4549	<0.0001
Albumin (g/dL)	3.47	3.55	3.63	3.80	0.00	<0.0001	0.1747	0.2862
Albumin/globulin, Alb/glob	0.93 ^{a}	0.99 ^{a}	0.82 ^{b}	0.95 ^{a}	0.01	<0.0001	0.0031	<0.0001
Total protein (g/dL)	7.30	7.20	8.15	7.84	0.10	<0.0001	0.0686	0.0674
Urea (mg/dL)	37.71 ^{a}	36.91 ^{a}	37.55 ^{a}	33.50 ^{b}	0.96	0.0103	0.0404	0.0185
Creatinine (mg/dL)	1.65 ^{c}	1.89 ^{b}	2.08 ^{a}	2.25 ^{a}	0.00	<0.0001	<0.0001	<0.0001
Aspartate aminotransferase, AST (U/L)	70.80 ^{a}	33.25 ^{c}	77.73 ^{a}	54.29 ^{b}	0.03	<0.0001	<0.0001	<0.0001
Alanine aminotranferase, ALT (U/L)	54.83 ^{a}	52.51 ^{b}	58.52 ^{ab}	59.41 ^{ab}	0.11	<0.0001	0.7309	0.0357
Alkaline phosphatase, ALP (U/L)	145.14 ^{a}	118.12 ^{b}	134.57 ^{ab}	119.03 ^{b}	5.69	0.1105	0.0527	0.0124
Sodium, Na (mEq/L)	139.03 ^{b}	142.00 ^{b}	147.97 ^{a}	145.77 ^{a}	1.18	0.7937	0.7937	0.0017
Potassium, K (mEq/L)	5.34 ^{c}	5.74 ^{b}	6.93 ^{a}	6.20 ^{ab}	0.00	<0.0001	0.0017	0.0004

Means on the same row with different superscript letters (^{a}, ^{b}, ^{c}) indicate significant effects ($p < 0.05$) of the interaction between sampling time (T) and genetic type (GT).

AST, ALT, and ALP are chemical indicators of tissue function: elevated levels of these enzymes occur when liver and pancreas are damaged. Significantly higher levels ($p < 0.05$) of AST were found in Apulo-Calabrese at exsanguination when compared with crossbreeds. It is interesting to note that the concentration of AST increased slightly in Apulo-Calabrese from T0 to T1, unlike in the crossbreeds which demonstrated a remarkable increase at slaughter compared with the values obtained at the basal level. According to Pugliese and Sirtori [21] local breeds are reared mostly in an extensive system where pigs forage for food in their surroundings. The elevated levels of AST found in Apulo-Calabrese at the basal condition could be a marker of an overworking hepatic metabolism due to their feeding with formula rations given to conventional fast-growing breeds. Nevertheless, the value obtained was within the range of values found in healthy pigs [38].

Despite the similar levels of ALT found in both genetic types of pigs at exsanguination, there was an increase in this parameter within both genetic types from T0 to T1.

The levels of sodium and potassium found in Apulo-Calabrese at exsanguination were higher when compared with crossbreeds and with values obtained at the basal condition. According to Mota-Rojas et al. [39] transport and slaughter can cause an increase in the concentrations of sodium and potassium, respectively. The values reported in this study were, however, within the normal physiological range for pigs [35].

The results of the PCA performed on the changes in blood concentrations between T1 and T0 are reported in Figure 1, where the score and the loadings plots are shown. Two samples were not included in this analysis since they appeared to be outliers in orthogonal distance plot. Multivariate analysis generated three Principal Components (PCs: PC1, PC2, and PC3) explained 30%, 14%, and 10% of the total variance, respectively. The two components that explained the most differences between the two genetic types were PC2 and PC3, since plotting these two components samples displayed to be clustered (Figure 1a). The score plot (Figure 1a) presents the graphical projection of the samples into a two-dimensional space with PC2 (t2 in Figure 1a) values as the x axis and PC3 (t3 in Figure 1a) as the y axis. The red and blue ellipses represent the Mahalanobis distances for the crossbreed and Apulo-Calabrese pigs, respectively, while the black ellipse showed the average area of Mahalanobis distances for the complete population. Figure 1b graphically displays PCA loadings, numerically presented in Table S2. In Figure 1b the variables weighting the most in each PC are displayed: variables which have little contribution to a direction (PC) have almost zero weight (like urea for PC3), while the ones that contribute the most in the definition of the PCs show higher or lower weights (like glucose for PC3). Therefore, the blood parameters that contribute the most in the explanation of the differences among crossbreed and Apulo-Calabrese pigs are grouped together at the opposite quartiles. The results obtained from the PCA were consistent with those observed in the mixed model. Interestingly, the variables that weighted most in PC2 were urea (0.431), AST (−0.406), alb/glob (−0.350), lactate (0.331), and ALP (−0.327) (Table S2), which were the blood parameters that were influenced by the time × genetic type interaction in univariate results. PC3 resulted to be mainly related to glucose (−0.587), which was influenced by the genetic type in the univariate analysis.

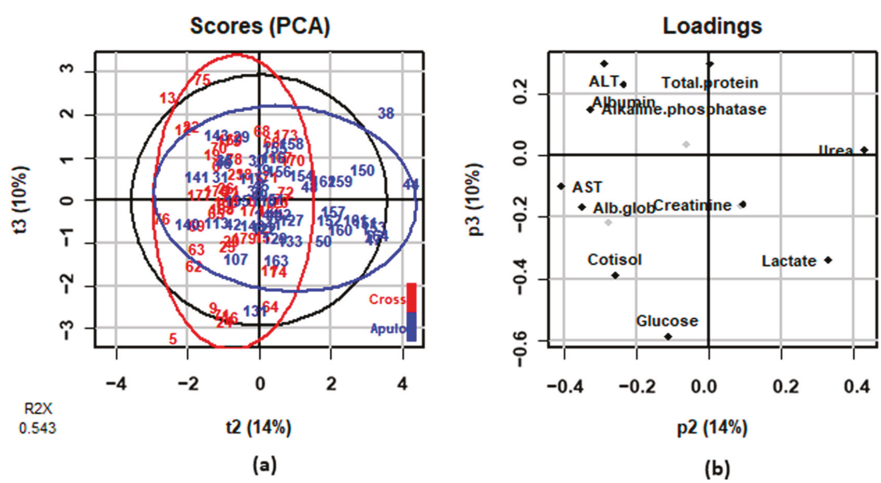

Figure 1. Results of principal components analysis (PCA) on the variations of blood parameters between T1 and T0: (**a**) score plots for principal component 2 (t2) and principal component 3 (t3) of Apulo-Calabrese (blue) and crossbreeds (red) samples; (**b**) loadings plot with the weights of variables included in principal component 2 (p2) and principal component 3 (p3).

3.3. Meat Quality Traits

The significance of factors of variations on meat quality parameters of the LT muscle is reported in Table S3. The interaction between measuring time and genetic type was significant for all colour coordinates and for shear force.

Post mortem pH decline (Figure 2a) was similar between genetic types although a significant ($p < 0.05$) decrease was reported in the first 6 h after slaughter, which stabilized during subsequent measurements. A similar pH trend was observed by Shen et al. [40] when comparing local Chinese breeds and crossbreeds of pigs.

L* (lightness) measured at 6, 24, 72, and 144 h after slaughter increased progressively as post mortem time increased (Figure 2b). The L* values measured in the meat of Apulo-Calabrese were significantly lower ($p < 0.05$) than those recorded in the meat of crossbreeds in all measuring times, with the exception of those recorded at 144 h after slaughter. The L* coordinate in the meat of crossbreeds stabilized after 24 h whilst that of Apulo-Calabrese maintained an almost constant increase. According to Scheffler and Gerrard [41] post mortem pH can affect muscle colour. Despite the similar levels of pH in this experiment significantly higher ($p < 0.05$) values of a* (Figure 2c) were found in Apulo-Calabrese at each detection time. This indicates that the meat from this breed is distinguished by a deeper red colour like other local European pig breeds [17,42,43]. The trend of the a* coordinate in Apulo-Calabrese pigs showed limited variations and the only value that was significantly different from the others was that measured at 24 h post mortem. The b* value did not differ between the two genetic types as shown in Figure 2d. For both breeds the highest b* values were recorded at 72 h post mortem, while the value decreased significantly at 144 h post mortem.

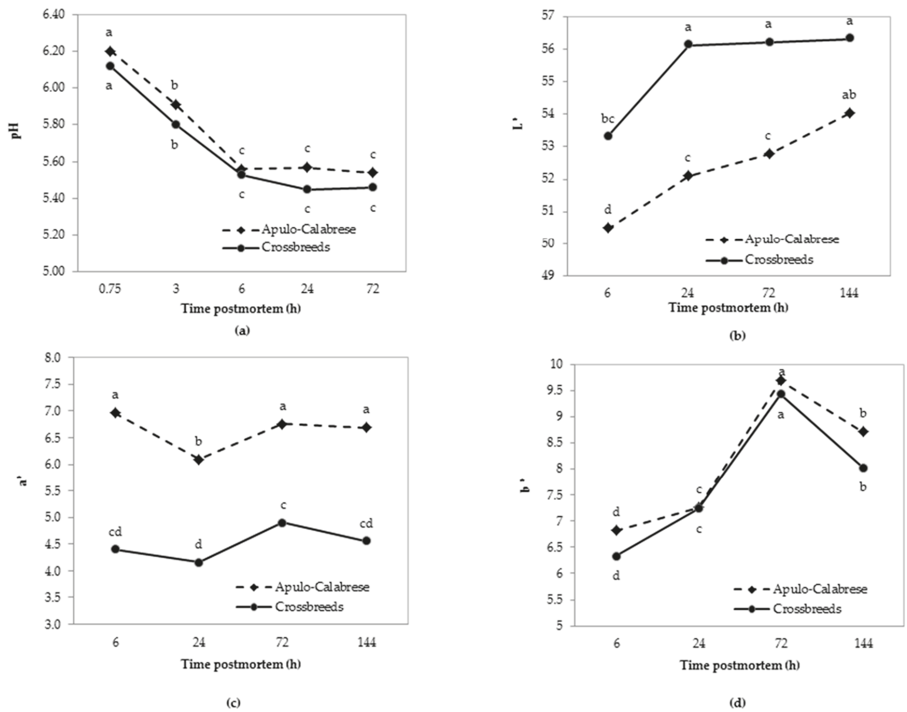

Figure 2. Changes in pH (**a**) and colour coordinates (L* in (**b**), a* in (**c**) and b* in (**d**)) in *longissimus thoracis* in Apulo-Calabrese and crossbreed pigs over time. Different letters in the graphs (a, b, c, d) indicate significant effects ($p < 0.05$).

Higher values of cooking losses were reported for both genetic types at 24 h post mortem (Figure 3a) compared to lower values recorded at 72 and 144 h after slaughter. Apulo-Calabrese pigs showed slightly higher values at 24 h post mortem compared to crossbreeds, but these differences did not reach statistical significance. Apulo-Calabrese showed lower values of cooking loss when compared with the values of other local breeds, like Cinta Senese [44] and Nero Siciliano [45]. There was no effect of genetic type on drip loss (4.19 ± 0.2 and 4.78 ± 0.2 for Apulo-Calabrese and crossbreeds, respectively).

Warner-Bratzler shear force measured after drip and cooking loss (Figure 3b) decreased as post mortem time increased and showed a similar trend in both genetic types. Nevertheless, higher values were reported for Apulo-Calabrese at 24 and 144 h after slaughter, suggesting the need to subject the meat of Apulo-Calabrese to ageing if it is intended for fresh consumption.

Figure 4 reports the results of the PCA performed on meat quality traits. Multivariate analysis generated four PCs: PC1, PC2, PC3, and PC4 explained 25%, 14%, 9%, and 8% of the total variance, respectively. The component that explained the most differences between the two genetic types was PC2, since samples displayed to be clustered for PC2 (Figure 4a). Figure 4b graphically displays PCA loadings, numerically presented in Table S4.

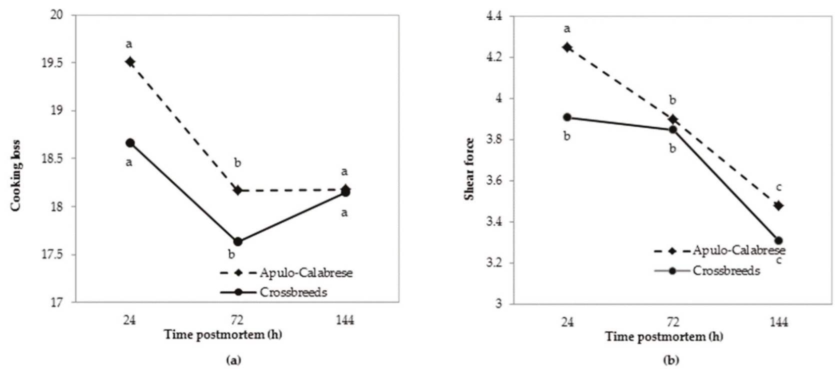

Figure 3. Changes in cooking loss (**a**) and shear force (**b**) in the *longissimus thoracis* in Apulo-Calabrese and crossbreed pigs over time. Different letters in the graphs (a, b, c) indicate significant effects ($p < 0.05$).

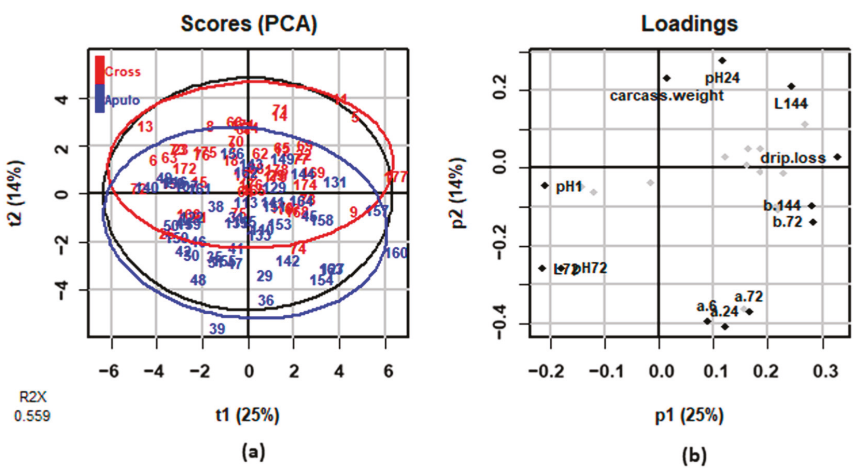

Figure 4. Results of principal components analysis (PCA) on the meat quality traits: (**a**) score plots for principal component 1 (t1) and principal component 2 (t2) of Apulo-Calabrese (blue) and crossbreeds (red) samples; (**b**) loadings plot with the weights of variables included in principal component 1 (p1) and principal component 2 (p2).

The variables that weighted most in PC2 were colour coordinates a* at 24 h (−0.409), a* at 6 h (−0.394), a* at 72 h (−0.368), a* at 144 h (−0.363), L* at 72 h (−0.257), and pH measured at 24 h (0.276). The high weights observed for a* colour coordinate are in agreement with the significant differences obtained from univariate analysis reported in Figure 2c, where redness-greenness value a* was highly divergent at all the measuring times between the two pig genetic types. Together with a*, other variables that contribute in differentiating the two genetic types were L* and pH, as can be noticed both by PCA loadings in Figure 4b and Table S4 and by mixed model results in Table S3. Interestingly, despite the different statistical assumptions of mixed and multivariate analysis, the results obtained are quite concordant, highlighting that colour coordinates represents the meat quality attributes discriminating the most the two genetic types. Anyway, PCA results suggested that, when considering together all the meat quality variables and taking into account their correlated nature, also pH measured at 24 h has a consistent weight in differentiating Apulo-Calabrese from crossbreed pigs. This result may also be noticed in Table S3, from the mixed model results. Despite the genetic type had not a significant effect on *longissimus thoracis* pH, the estimated L.S.M. for pH at 24 h were the most divergent between the two genetic types (5.57 for Apulo-Calabrese and 5.45 for crossbreeds) when compared with the pH measured at the other times. This result suggests that using a combined statistical approach may allow to highlight the main differences that would have not been appreciable with the use of univariate statistics alone.

4. Conclusions

Overall, the results obtained in this study broaden the knowledge on the Apulo-Calabrese pig breed, which showed higher levels of lactate, urea and AST after short distance transport indicating a more intense physiological response when compared with crossbreeds. With regards to meat quality, similar trends for pH, drip loss and cooking loss were found for both genetic types. The higher a* coordinate found in Apulo-Calabrese pig indicates that meat from this breed has a deeper red colour and can be used for the production of typical cured meat which on the basis of the gathered evidence could be produced without the use of additives intended to improve colour. The results in this preliminary study may provide insight into the response of local breeds to the transport process. However, due to practical restraints, in this pilot research it was not possible to investigate the effects of different pre-slaughter treatments on the behavioural and physiological responses of the two genetic types, further research is needed to evaluate the effect of different transport durations and handling practices on welfare and meat quality traits when transporting local pig breeds.

Supplementary Materials: The following are available online at at http://www.mdpi.com/2076-2615/8/10/177/s1. Table S1: Percentages of Apulo-Calabrese and Crossbreed pigs showing the different behaviours during loading, unloading, and lairage with the P values of the differences between percentages; Table S2: Loadings of the PCA performed on blood parameters; Table S3: Significance of factors of variations included in the model used for meat quality traits; Table S4: Loadings of the PCA performed on meat quality traits.

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animals

Review

Mobile Poultry Processing Unit as a Resource for Small Poultry Farms: Planning and Economic Efficiency, Animal Welfare, Meat Quality and Sanitary Implications

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Simple Summary: Poultry meat production is now based on fast-growing strains, with consequences for animal health and welfare. There is also an increasing demand for products from extensive rearing systems; there are, however, several criticisms including the difficulty of slaughtering chickens from a logistic, legislative and economic point of view. A possible solution could be represented by the use a Mobile Poultry Processing Unit (MPPU), which directly reaches the poultry farms. The aim of this review is to analyse the requisites and economic efficiency of a MPPU prototype in Italy; further, the related animal welfare aspects and the qualitative and sanitary implications are discussed.

Abstract: Nowadays there is an increasing demand for poultry products from alternative rearing systems. These systems, commonly named pastured poultry production (PPP), are more expensive than intensive rearing system but sustain biodiversity, local economies and farm multi-functionality besides providing meat to which consumers attribute high ethical value and quality. PPP generally uses large outdoor runs, small number of animals and requires chickens adapted to natural environment. One of the most relevant obstacles to further development of PPP systems is related to the slaughtering of animals economically and at the same time complying with the sanitary regulations to maintain food safety standards. A possible solution could be represented by a Mobile Poultry Processing Unit (MPPU), which directly reaches the poultry farms. MPPU can consider a good compromise for the niche production providing an opportunity to small farmers to exploit the full potential of their production system. The aim of this review is to analyse the essential requisites and MPPU economic viability in an Italian system. Qualitative, societal aspects are discussed together with bird welfare and hygiene implications. The case study indicates the viability of MPPUs but notes that up scaling to medium sized operations would not be permissible under current EU regulations.

Keywords: Mobile Poultry Processing Unit; pastured poultry production; animal welfare; meat quality; economic efficiency

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1. Introduction

Poultry production has a lower environmental impact when compared to other livestock production chains [1], mainly due to its high efficiency in converting feed into meat. The main reason for this high efficiency ratio is related to the strong genetic selection carried out to increase productive performance. Modern broilers reach their slaughter weight earlier than ever before, with a high yield of breast and other meat [2]. Unfortunately, these fast-growing strains can show welfare and health problems, skeletal imbalance [3], metabolic disorders, myopathy and other muscle disorders [4], which affect the appearance of the meat, nutritional traits and consumers acceptance [5].

A side-effect of this process is the reduction of genetic variability [6] and the vulnerability of these chickens to environmental stress [7]. Nevertheless, poultry meat production of western countries is based on these chicken strains and it is now accompanied by a growing concern for the health and welfare of these animals [8]. As a result, there is an increasing demand for poultry meat produced in extensive rearing systems [9]. These production systems, commonly named pastured poultry production (PPP), are more expensive than intensive systems but can help sustain biodiversity, local economies and farm multi-functionality, in addition to providing meat to which consumers attribute a high ethical value, quality and taste [10]. Indeed, PPP generally uses large outdoor runs (at least 4 m^2/chicken), small number of animals and requires chickens adapted to a variable environment, without the strict control of temperature, humidity and ventilation of in the intensive systems [11].

It is widely known that the access to free-range areas greatly improves the welfare of poultry and that the presence of shrubs and trees in the pastures further increases the use of runs [12]. For these reasons, the PPP is often found in an agro-forestry production system (such as fruit orchards or olive orchards). This combination improves the environmental sustainability of production because two or more different productions (meat, fruit and crops) can be simultaneously obtained from the same land, providing advantages both for the chickens and for the orchard.

Chickens and orchards mutually benefit each other; chickens improve soil quality by adding organic matter and control both insect pests and weeds, while trees protect the chickens from adverse weather conditions as well as raptors and provide additional revenue for the farm [13].

Even though the pasture offers only a modest supply of energy and proteins—the pasture in PPP represent only 10–15% of the total feed intake—it provides many bioactive compounds, such as xanthophylls, antioxidants and vitamins [14,15]. Accordingly, the meat from PPP may have some nutritional benefits compared to standard broiler meat [16]. Several authors [17,18] reported that bioactive compounds are transferred from the pasture to chicken meat, as shown by a higher meat content of antioxidants and n-3 polyunsaturated fatty acids. Additionally, access to pastures may contribute to meat flavour, with some forage and herbs resulting in distinctive flavours [19,20].

As previously reported, PPP requires chickens that are adapted to the natural environment (high kinetic activity and foraging behaviour), with a well-developed immune system and adequate body conformation and skeletal development [11,21–23]. Previous research has shown that slow-growing chickens are more adaptable to outdoor runs due to suitable thermo-tolerance, foraging aptitude, immune response and antioxidant capacity compared to fast-growing strains [24–26]. In contrast, fast-growing broilers selected for intensive production systems are fit for living in controlled conditions (controlled environment, veterinary care and diets high in protein and energy) and do not adapt to PPP. Dal Bosco et al. [10] compared behaviour in the chickens and showed that slow-growing chickens covered an average daily distance of 1130 m, while fast-growing ones walked only 220 m.

Contrary to the popular belief, there are several disadvantages with respect to PPP that preclude further development and reduce the production capacity of this system. The main disadvantages are reported below:

Cost of production—The production cost of this meat is much higher than with a conventional system, mainly due to the lower growth performance and the breast meat yield of slow-growing genotypes.

Risk of predation—PPP systems are attractive to predators (foxes, birds and other wild animals). Permanent fencing is expensive and is not always effective at excluding predators from the pasture.

Rules formulated for large-scale poultry farms—Sanitary rules and technical standards of poultry production are often based on large-scale farms. Small farmers cannot afford to invest in requirements and protocols unsuited to their system of production [27].

Consequently, up and down-stream infrastructures (e.g., hatchery with genetic strains adapted to PPP or slaughter houses), services (know-how or vaccines for small number of birds) are lacking. In particular, one of the major bottlenecks is the slaughterhouse. During the last 20 years in western countries, there has been a huge decrease in the number of poultry slaughterhouses coupled with an increase in size of the existing ones. Moreover, nowadays slaughterhouses generally belong to food companies and are not available to other farms.

The lack of slaughterhouses strongly discourages the use of PPP and many farmers limit their production to less than 500 or 1000 birds per year. This quantity, according to European (853/2004) and USA (62) regulations, is considered to be primary production which can be sold directly to consumers without controlled slaughter. However, the restrictions on sales and the lack of small-scale poultry slaughterhouses prevent the creation of specialised poultry farms. Small-scale farmers who wish to sell poultry products locally must have them slaughtered and processed in inspected facilities that are usually far from the farms.

A possible solution which increases the use of PPP systems could be a Mobile Poultry Processing Unit (MPPU) mounted on a small truck or van which goes directly to the poultry farms. A MPPU is excluded from continuous inspections by the Food Safety Authorities but it is still required to meet all sanitation and requirements. These MPPUs are designed to eliminate regulatory impasses and increases marketability and profitability for small-farmers. The MPPU reaches the farm directly on the day of slaughtering and then another advantage is the absence of transport of live animals to the slaughterhouse. This positively influences animal welfare and meat quality (see Sections 5 and 6).

The main characteristics of MPPU are described later in the Sections 2 and 6 and pros and cons are summarized in Table 1.

Table 1. Main characteristics of Mobile Poultry Processing Unit vs. conventional slaughterhouse.

Aspects Considered	MPPU		Conventional Slaughtherhouse	
	Pros	Cons	Pros	Cons
Essential Requirements	Small truck easily handled	Need of a site for slaughtering in the farm (H_2o, electricity, etc.)		
		There are numerical and geographic restrictions	No numerical and geographic restrictions	
Operational and Economic Efficiency	Sharing the MPPU by farmers allows to reduce the processing cost	Low number of birds processed/hour	High number of birds processed/hour	High unitary cost for transporting live animals (farm–slaughterhouse)
	Possible use of a refrigerated trailer for the delivery of slaughtered carcasses			Cost for transporting carcasses (farm-market)
	Public funds can partially cover MPPU purchase			No public funds for the purchase
Animal Welfare	No chicken transport and reduction of pre-slaughter stress			Transport negatively affects animal welfare
Qualitative and Sanitary Implications	Safeguarding the quality of meat (low stress)	Good Manufacturing Practices (GMP)for welfare respectful handling and limited time crating		Pre-slaughter stress negatively affects meat quality: change in colour, shelf-life, nutritional parameters
	Low risk of cross contaminations (one flock per day)			Cross contamination due to the processing of different batches of flocks per day Reduction of hygienic/safety condition of meat due to the pre-slaughter stress.

Table 1. *Cont.*

Aspects Considered	MPPU		Conventional Slaughterhouse	
	Pros	Cons	Pros	Cons
	Reducing carcass contamination due to crating and transport	Difficult in biosafety management (specific for pastured poultry production) Higher age of the birds (Campylobacter)	Proper biosafety measures at flock level	GMP for handling, crating and transport (particularly from different flocks in the same day)
		No decontamination strategy applicable (not allowed in European Union)	Possibility of decontamination strategies of the carcass (water bath chilling)	

Beside the economic aspects, there are also social aspects connected with the use of PPP and MPPUs. In a sound productive chain, the system of production should be followed by a coherent transfer of goods and services (from farm to consumer). Each productive system should develop a proper system. For example, intensive poultry production has developed not only a proper production system but also a typical structure for exchange. Accordingly, standards arising from industrialised agriculture may be at odds with the principles of small-scale poultry systems [28].

Rural sociology suggests a general framework in which exist a correspondence between production systems and the structure for exchange. PPP, as other local production system, requires the creation of specific short and decentralised circuits that link the production with the consumption of food. This pattern is completely different from highly centralised paths constituted by large food processing and trading companies which operate on a large scale [29].

Our opinion is that the exchange structures promoted by industrialised processing may not be able to handle all the benefits of PPP and they could negatively influence its internal equilibrium [29]. Conversely, PPP is not able to exploit the benefits of the large-scale supply systems. Accordingly, PPP requires specific approaches to solve marketing issues; these approaches cannot be derived from the experience of other productive systems.

According to this view, a MPPU could be considered not only a solution to the slaughtering problem but also a resource to enhance the emergence of food circuits (e.g., farmers' markets and niche markets), which cannot feature within the global food chains [30–32]. Indeed, a MPPU can contribute to managing this emerging circuit, allowing the creation of a production chain which exploits the ethical value adapted to its dimension value, prize.

Moreover, through use of a MPPU, the cost of each farm owning a slaughterhouse (€50–80,000) is avoided. European legislation recognises and authorises on-farm slaughter but the slaughterhouse must be used only for animals raised on the farm. This causes high initial investment for each farmer. In the light of what is reported, the aim of this paper is to review the current MPPU technologies by examining:

- Essential requirements of a MPPU
- Operation and economic efficiency
- Animal welfare aspects
- Qualitative and sanitary implications.

In the USA, several types of MPPU have been available for about 10 years [33,34], whereas in Europe, mobile slaughtering facilities are rare and only a few data are available. Accordingly, the present review will focus mainly on EU regulations and the EU situation.

2. Essential Requirements: Planning and Layout

During the last 10 years, National Authorities have pointed out rules to allow the activity of these MPPU. The basic principles of slaughtering in the EU include the need to fulfil several requisites, specified in the Regulation EC/853/2004 (Annex II, Chapter I, III and IV point 1) and by the Sanitary Authority which eventually authorises the use of the MPPUs on different farms. In this way, it is possible to share the MPPU among several farms, thus reducing the slaughtering cost of each chicken.

Furthermore, the structure needs to be properly designed to avoid cross-contamination and it should be placed in a specific area of the farm where drinking water and electric power are available (unless a drinking water tank and a generator are present to directly supply the MPPU), regular pest control is performed and waste (water and slaughter by-products) can be easily managed.

The MPPU must be built with materials and equipment that are easy to clean by Sanitation Standard Operating Procedures (SSOP) and there must be adequate site management and appropriate personnel hygiene and clothing. Personnel operating in the MPPU must be trained in slaughter procedures and a proper HACCP (Hazard Analysis and Critical Control Points) plan needs to be implemented in the MPPU.

With regard to the slaughter procedure, stunning, bleeding and plucking must be performed separately from evisceration. Stunning must be performed according to Regulation EC/1099/2009. Chilling and storage of carcasses and meat must take place immediately in the MPPU or on the farm. If chillers, generally static chillers, are available on the farm, they must be placed near the MPPU and a suitable system for protecting the carcasses during transport from the slaughter site to the chiller needs to be adopted to avoid exogenous contamination.

Animal by-products must be managed according to Regulation EC 1069/2009. Cleaning and disinfection of the MPPU must be performed at the end of each slaughtering session, eitherat the farm or in a specific staging area. Meat must be labelled with the date of slaughter, farm code and farm address. A proper traceability system has to be set up.

Not all poultry farms have these facilities and an evaluation of the best site on the farm for slaughter, supply of water and management of wastewater, is needed.

In the United States, the hygiene requirements and SSOP are almost the same as in the EU (Mobile Poultry Processing Unit Farm & Food Safety Management Guide, 2012). Although the local area is considered as the State and the maximum number of birds slaughtered is higher than 10,000.

The other restrictions imposed by law in the EU are:

- The MPPU can be used on poultry farms producing less than 10,000 birds per year (EU State-Regions) [35];
- The meat processed in a MPPU can only be sold in neighbouring areas (the province where a MPPU is located and adjacent areas, within 50 Km of the province border) and this is not valid for all States of the EU;
- The buyer should be a retailer, that is, selling directly to the consumer.

According to this set of rules, production from a MPPU is small, local and subjected to severe numerical and geographical restrictions, confirming this system as a close and small one.

At the short term, the geographical and numerical limitations imposed by EU regulations do not permit future development of MPPUs for medium- or large-scale production. Moreover, even if some technical (different stunning system, carcass decontamination and water bath chilling) or regulatory improvements (geographical limitation) of MPPUs were advisable, European legislation strictly limits further improvements. Indeed, the change of regulatory system, at least in EU, is very long and complex and needs multiple level of decision (European, National and National/Regional).

Nonetheless, other countries could take advantage of the European experience and improve MPPUs according to national legislation.

3. A Case Study of MPPU in Italy

According to these requirements, a MPPU has been planned and built in central Italy (Figures 1 and 2). This type of MPPU is the first in Europe and for this reason the study was carried out considering only whit this equipment.

The small dimension of this MPPU has made it possible to put the equipment in a small truck, which can be handled with a standard driving license. It is also possible to add a refrigerated trailer for the farmer to deliver the carcasses directly to the market after slaughtering.

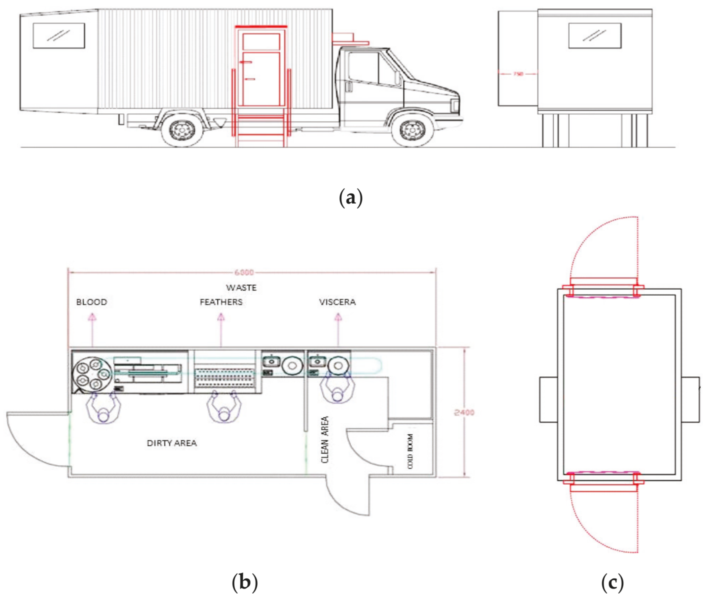

Figure 1. Schematic layout of the Mobile Poultry Processing Unit (50 chickens/hour): external (**a**) and internal (**b**) configuration and refrigerated trailer (**c**).

The innovative point of this process consist in the absence of animals transport, in fact the MPPU reaches the farm the day of the slaughter improving the pre-slaughtering operations. The slaughter process begins by a withdrawing feed of 8–10 h and a sanitary control of animals. One hour before slaughter, all of the chickens are captured, caged and placed on the MPPU's external platform (Figure 1a).

The truck is internally divided into two areas as required by the regulations:

- Dirty area;
- Clean area; so at least two operators are needed, one for each area.

The employee in the dirty area carries out all the operations from stunning to plucking; in particular he takes the animals one at a time from the cage, electrically stuns them and places them in the bleeding cone.

The subsequent phases involve the processing of four animals at a time that are placed in the scalder at a temperature of 58–60 °C for 30–60 s to loosen the feathers and in a drum plucker for 40–50 s.

At the end the carcasses are hung in hooks and transferred to the clean area where the second operator provides to the eviscerating. The final two steps are represented by the refrigeration and packaging. The working capacity of this MPPU is about of 50 chickens/hour.

Figure 2. Photo image of a MPPU.

4. Operational and Economic Efficiency

Since a MPPU requires substantial investment by the farmers, an evaluation of the economic feasibility of a MPPU compared to the current processing methods would allow farmers to plan their production method [34].

Different MPPUs—from basic to more automated and expensive ones—have been developed worldwide, mainly in the USA. Naturally, the cost of the equipment can vary substantially (from about €10,000 to 180,000).

Starting from the case-study shown above, we calculated the cost of chicken processing (Figure 3). In our case-study, the MPPU was designed for a small truck (IVECO DAILY; l 5000 × w 2200 × h 2400 cm), whereas the dimensions of the refrigerated van were l 3240 × w 1550 × h 2105 cm.

The equipment for the slaughter of poultry was separated into two areas: "dirty area" and "clean area" (Figure 1). The "dirty area" comprises a platform for live animals, an electro-narcosis stunner, kill tank and cones, scalder, steriliser for knives, plucker and carcass guideway. The "clean area" comprises an evisceration table, a semi-automatic eviscerator, a steriliser for knives, a fridge, a table with a weighing scale and a generator.

As required by official controls on food safety, at least two people have to work in the MPPU, one in the clean area and the other in the dirty area. The number of birds/hour which can be processed is around 50.

The total cost of the equipment (truck + van) is roughly €150,000, which can be shared among different farms. In a case scenario of 10 farms, the cost would be reduced to about €14–15,000 per farm, compared with a cost of about €50–80,000 for a farm slaughterhouse.

In addition, the MPPU cuts out the costs related to the transport of live animals from the farm to the slaughterhouse and the subsequent transport of the refrigerated carcasses back to the farm or stores. Typically, the transport of live animals and carcasses must be carried out by different vehicles, the first one complying with the regulations concerning animal welfare, the second one complying with the hygienic-sanitary requirements for meat which mainly involves refrigeration.

It should also be taken into consideration that EU and National funds, which support the improvement of competition and the modernization of farm facilities, could partially cover (30–50% of the eligible cost) the purchase of a MPPU.

It should be underlined that the avoidance of transportation permits to improvement in the welfare of chicken, see the next section.

Naturally, the care of animals during catching and caging is also particularly important in a MPPU. To avoid excessive chicken shackling, the chickens should be caged just before slaughter. Caged animals are placed in the rear platform of the truck equipped with a cover, which serves as a rest area where the veterinarian can perform the pre-slaughter inspection of animals.

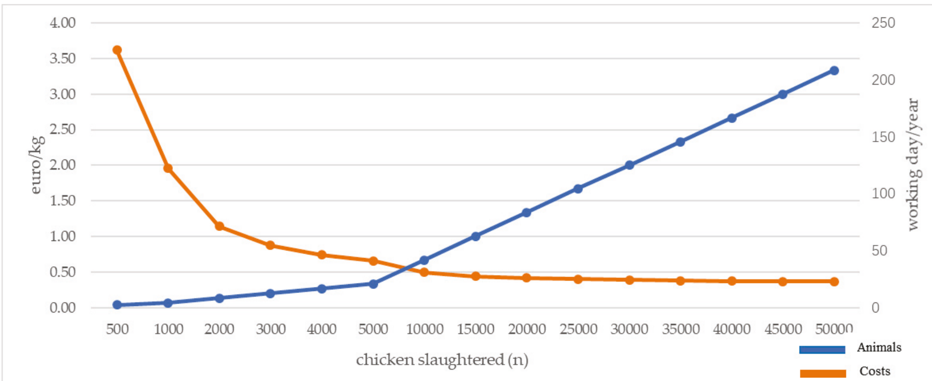

Figure 3. Slaughtering cost (€/kg) and number of working days according to the number of chickens slaughtered (our elaboration).

These costs are based on an actual Italian case study. According to Figure 3, when the number of chickens slaughtered is very low (<2000 per year) the cost is high (>€1/kg live weight processed), whereas the trend is for the cost to become consistently lower for numbers > 25,000. Accordingly, small farmers should efficiently plan the number of chickens to be slaughtered, with the possibility of collective use of the same MPPU. Other management options are also available (e.g., leasing and rent) and can make the use of MPPUs less expensive.

Angioloni et al. [34] showed a similar trend (decreasing costs with increasing number of slaughtered chickens) and showed that ten farmers sharing the ownership of the MPPU can achieve a higher profit than using alternative off-farm inspected slaughter facilities. In the USA, current estimates show that the cost is variable but within a close range.

The cost of conventional slaughter includes:

- transport of live animals by authorised vehicle €1–1.50/chicken (depending on the distance of the slaughterhouse from the farm);
- slaughtering process 0.50 €/kg of live weight, therefore, an average of €1.50/chicken;
- transport of carcass by refrigerated vehicle, €1.50/carcass.

In summary, the cost of slaughter for each animal ranges from €3.50 to €4.50.

In conclusion, the economic analysis indicates the cost of slaughter using a MPPU is, on average, on the same scale as the cost of an on farm stationary processing system when the number of animals is higher than 10,000 year (the maximum admitted for this stationary plan) and lower than costs involved with a commercial slaughterhouse.

5. Animal Welfare Aspects

As previously reported, slaughterhouses are becoming bigger and the distance between farms and slaughterhouses is, in some cases, very large.

European law sets out several compulsory requirements on the transfer of poultry to slaughterhouses: density in the crates; allowing drinking and feeding if more than 12 h are needed to reach the abattoir; limits to faecal matter falling from animals in upper layers to the underlying crates; and temperature and ventilation in the trucks during transport. These rules mainly aimed at fast-growing broilers produced on intensive farms. However, very little data are available with respect to transport of birds from free-range systems [11]. It is expected that the more active animals used in PPP systems will respond to this transport stress differently to fast-growing chickens.

Poultry transport to the slaughterhouse is one of the critical factors affecting animal welfare, quality and meat hygiene. These different stressing situations can reduce bird welfare and increases the risk of body injuries (broken wings/legs and overall distress) and mortality [36]. Chickens are caught and placed in crates to reach the slaughterhouse and during the transport they have no feed and water, are exposed to environmental changes (i.e., movement, noise, vibration), subject to even extreme conditions of temperature and humidity, forced to counteract the track movement [37].

Many studies have focused on the effects of transport stress on different blood traits [38,39]. Zhang et al. [40] reported that transportation of broilers caused decrease in glycogen in breast and thigh muscles. In addition, transport stress is associated with enhanced skeletal muscle energy metabolism, causing mitochondrial superoxide production, acceleration of lipid peroxidation and the induction of cellular damage [41]. In chickens, stress and kinetic activity before slaughter are also involved in pH variations during the early stages of rigor [42], whereas the final pH of meat mainly depends on the glycogen content at the time of slaughter [43].

Thus, time spent in transit to the slaughterhouse is a major concern in terms of welfare and meat quality. The effect of transport duration on animal welfare and the resulting meat quality in broilers have been well researched but data on the interaction between genetic strain and transport duration are sparse. It has been reported that the effect of stress could be different in fast- and slow-growing poultry strains [44].

Fast-growing strains tend to produce meat with a slower pH decline, higher ultimate pH and, consequently, greater water-holding capacity [45]. On the other hand, Berri et al. (2007) [46] reported that slow-growing strains suffer more from the lag-phase between catching and slaughter due to their high kinetic activity (i.e., wing flapping) during transport and slaughter. Accordingly, when broilers are subjected to stressful conditions, 'have been well researched could be different from that in standard broilers.

Our previous results [47] suggest that a 4-h journey to the slaughterhouse, compared to immediate slaughter in a MPPU, negatively affects some animal welfare traits (tonic immobility, creatine kinase, heterophil/lymphocyte ratio, lysozymes, reactive oxygen species, glucose and haptoglobin) in free-range chickens. The slow-growing chickens showed the highest susceptibility to stress, even with a greater antioxidant defence due to their foraging behaviour. Accordingly, a less stressful slaughtering procedure should be developed for all chicken strains with shorter resting times in the farm, transport and animal storage at the slaughterhouse. This is particularly important for PPP in order to sustain the high welfare standard achieved during life and to maintain meat quality.

6. Qualitative and Sanitary Implications

The introduction of a MPPU could have an impact on the quality and hygiene/safety traits of the meat based on three main paradigms: reduction of pre-slaughter stress, transport procedures and proper implementation of the slaughter process (i.e., well-managed small-scale facilities, small number of animals of the same flock slaughtered per day).

Currently, it is understood that that the reduction of pre-slaughtering stress, especially catching, crating and transport, could affect meat traits. The increased level of epinephrine and glucocorticoids in animals exposed to *ante-mortem* stresses can affect *post-mortem* metabolism and, therefore, meat quality [48]. Pre-slaughtering stress, in particular due to transport, may increase muscle glycogenolysis resulting in glycogen decrease in both breast and thigh muscle [40]. Furthermore, acceleration of lipid peroxidation and induction of muscular cellular damage have been reported after stressful transport, associated with enhanced skeletal muscle energy metabolism and mitochondrial superoxide production [41].

Despite there is not a general consensus, these stressful events could therefore affect conversion of muscle to meat and the related protein functionality, following a reduced consumer acceptability and processing functionality of the meat caused by the changes in the water holding capacity, colour, tenderness, texture and shelf-life of meat and derived products [49]. Thigh meat have been reported to be affected more than breast meat by this phenomenon [50].

As previously reported, studies on the effects of pre-slaughtering practices on meat quality have mainly been conducted in fast-growing broilers, where muscle abnormalities (PSE—Pale, Soft and Exudative and DFD—Dark Firm and Dry condition) were also recorded but when slow-growing strains were considered, they seemed more subjected to stress than fast-growing genotypes due to high kinetic activity during catching, transport and wing-flapping during slaughter [43]. Castellini et al. [51] evaluated the effect of transport duration (0 h vs. 4 h) and chicken genotype (fast- vs. slow-growing strains) reared under free-range conditions. They observed that transport affected the fatty acid profile of breast and drumstick muscle, with a decrease in polyunsaturated fatty acids and antioxidant content (α-tocotrienol, α, δ-tocopherol and carotenoids) and an increase in TBARS (Thiobarbituric acid reactive substances) in breast meat (Figure 4). The decrease in γ-tocopherol, retinol and TBARS was more relevant in birds that were more active, probably due to the higher kinetic activity and the higher peroxidability of their meat. Furthermore, in this study, the breast muscles from 4 h-transported chickens showed significantly less lightness, and also meat tenderness (shear forcevalue) was affected by genotype and transport: meat from slow-growing birds was tougher, whereas after transport, in both genotypes, higher tenderness was observed. Nevertheless, neither PSE nor DFD were recorded.

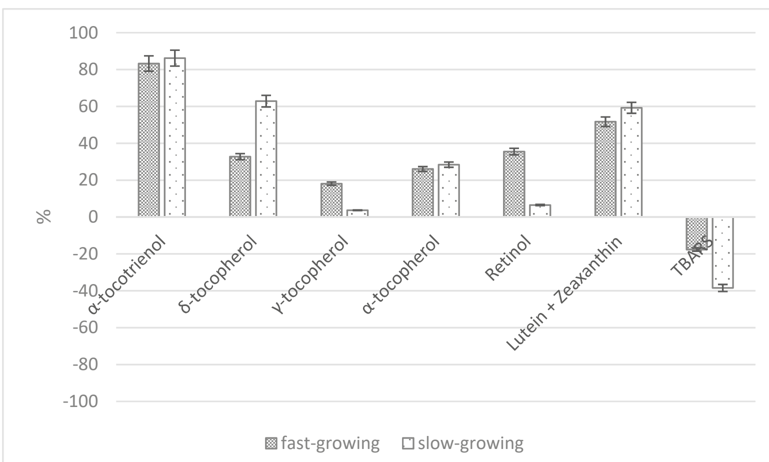

Figure 4. Variation (% with respect to no transport) of antioxidants (α-tocotrienol, α-, γ-, δ-tocopherol, retinol and carotenoids) and TBARS in fast- and slow-growing chicken strains after 4 h of transport (modified by [51]).

A PPP system together with a MPPU, when slow-growing strains are used and reduction in the number of chickens to be caught and slaughtered, combined with the absence of transport limits the time spent struggling in crates and, therefore, improves/preserves meat quality.

From a hygiene point of view, there is a large consensus that pre-slaughter stress increases the spread of infectious diseases [37]. The stress that birds experience during pre-slaughter procedures can enhance colonisation by *Campylobacter* spp. [52] and its spread throughout the flock [53].

Previous thinning of the flocks was considered as a major risk factor for contamination of chicken carcasses with *Campylobacter* spp. at the slaughterhouse and catching of the birds for crating further increases *Campylobacter* spp. contamination [54].

In addition, transport vehicles and crates can be considered to be a source of *Campylobacter* contamination [55]. *Campylobacter* from the processing plant can survive on crates for a period sufficient to contaminate the majority of farms in the catchment (it survives for at least 3 h after sanitisation) [56] and poses a contamination risk for uninfected birds belonging to other unrelated flocks [57,58]. Reduction in the time that animals spend in the crates and limiting slaughter to a small number of animals per day that could be caught without prolonged struggling, as well as the absence of transport, could improve the hygiene level of the carcasses.

With regard to *Salmonella* spp., environmental stress could weaken the immune response of birds, with an increase in number of pathogens on the crates [59]. For this reason, reduction in the handling procedures and the absence of transport, as with a MPPU, could strongly influence the prevalence of pathogens at the slaughterhouse. GMP (Good Manufacturing Practices) or guidelines on operator behaviour during pre-slaughter steps could be useful for informing producers about correct handling and crating procedures (with regards to timing, animal density and welfare) to be adopted in MPPU.

Nonetheless, it is reported that the older age of the animals at slaughter, generally adopted in PPP, increased the contamination of caeca by *Campylobacter* spp. [60,61]. Furthermore, when the prevalence of infected animals in the flock is high (i.e., slow-growing genotype with a relatively longer period of rearing), no reduction in *Campylobacter* spp., even without transport, were observed [47].

The same consideration was not so for *Salmonella* spp., as different authors reported no shedding animals and no positive carcasses in PPP systems and MPPUs, respectively [47,62].

With regard to the slaughter practices in a MPPU, all the procedures are carried out on a manual basis instead of using industrial-scale, automated commercial processing lines [62]. Furthermore, differences in the structures and equipment adopted, as well as in the procedure implemented may strongly affect the hygiene level of the carcasses. For example, in Europe the decontamination strategies could not be used and the limited space available in the truck reduce the possibility of using water-bath chilling with chlorinated water.

Reports on the effect of a MPPU on sanitary traits in poultry meat are scarce [62,63]. It seems likely that the slaughter of a single homogeneous batch of chickens from the same flock during one-day operations could reduce the cross-contamination reported when animals come from different batches and flocks to the same slaughterhouse [54] and, therefore, a daily slaughter rotation of the flocks with a properly cleaned and disinfected MPPU is strongly suggested [64].

Other specific aspects on the possible contamination route inside a MPPU are dependent on structure and equipment. Scalding, defeathering, evisceration and chilling are considered to be the major routes of contamination by both *Salmonella* and *Campylobacter* spp. [65] and have to be carefully considered during HACCP implementation in a MPPU.

In particular, due to the limited space inside a MPPU, chilling could be carried out in two steps (pre-chilling and chilling) which could be performed within the MPPU and in the farm, respectively [66]. This could have the advantage of allowing the MPPU to be cleaned and disinfected immediately after slaughter, while chilling and storage of carcasses are performed in the farm.

The use of an air chiller could be more practical for a MPPU, even if counter-flow water-chilling and decontamination strategies, when allowed by national legislation, could be more effective in reducing carcass contamination [62,65]. High carcass density in the chiller could also be avoided to allow proper chilling of the meat and reduce cross-contamination between carcasses [54,67].

In the USA, a technical survey on *Salmonella* and *Campylobacter* showed that *Campylobacter* prevalence was significantly higher in MPPUs and this was partly due to wastewater and compost. In view of this, the processing of waste should be improved for optimum control of human pathogens.

In Europe, animal by-products must be disposed of as quickly as possible to avoid contamination of the meat for human consumption (Regulation EC 1069/2009), thus providing proper protection of the environment from food-borne pathogens.

The prototype of MPPU shown here was provided by a detailed HACCP manual, with a risk assessment based on hazard probability and severity at each step of the process, validated during the first three months of slaughter and after one year of activity. One of the operators of the MPPU should be responsible for the HACCP plan, including SSOPs. Cleaning and disinfection of the truck and equipment and assessment of the risk of carcass contamination due to scalding, defeathering and evisceration steps (GMPs) and carcass chilling, as the real CCP (Critical Control Point) able to prevent the growth of pathogens, also have to be taken into consideration.

The absence of *Salmonella* on the carcasses, as well as counts of *Campylobacter* spp., following the criteria lay down by EC Regulation 2073/2005, could be adopted in a MPPU as evidence of the hygiene level, as already performed in conventional industrial slaughterhouses. A reliable carcass sampling could be planned, according to EC Regulation 2073/2005, with 50 samples which should be derived from 10 consecutive sampling sessions

Place and day of slaughtering must be provided to the veterinarian officer to permit Official controls of MPPU.

7. Conclusions

MPPUs are designed to eliminate regulatory impasses and increase marketability and profitability for small-farmers. In addition to economic and technical aspects, there are also other ethical aspects connected with the use of MPPUs.

Nevertheless, positive conclusions exist concerning the effect of MPPUs on animal welfare and product quality. However, inconsistent findings are available regarding sanitary aspects due different equipment and procedures. However, considering that this system practically removes the need to transport poultry and it is used for small quantities of chickens, it is expected that the sanitary aspect can also be improved.

The MPPU could be judged a first step in the development of a new model of alternative poultry production, because it favours different types of change, from farmer to consumers and between the individual stakeholders. In Italy the difficulty to slaughter a low number of animals negatively affect the local productions. The MPPU beyond that to improve the marketing products could increase the connection between the small farms. Developing a network starting from the sharing of the MPPU could ameliorate the collaborations among small farms.

Concerning the Italian poultry sector, the farms have lost her entrepreneurship for the presence of the big companies that control all the production chain.

According to this view, MPPUs can be considered not only as a solution but also as a resource to emphasise the emergence of food circuits (e.g., farmers' markets and niche markets) which cannot feature within the global food chain.

Despite the improvement in poultry welfare (no transport or limited period of transport), the geographical and numerical limitations imposed by EU regulations mean that MPPU development for medium- or large-scale poultry production is unlikely to occur. European legislation has limited further improvement of MPPUs (e.g., different stunning system, carcass decontamination and water-bath chilling), confining PPP meat to local production and selling. Nonetheless, other countries could take advantage of the European experience and improve MPPUs according to national legislation.

In developing countries where the demand for livestock products is strongly increased and in many case the society is organized in small and poorly connected units, the MPPU could represent a real and feasible opportunity of progress.

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Article

Use of Foaming Disinfectants and Cleaners to Reduce Aerobic Bacteria and Salmonella on Poultry Transport Coops

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Simple Summary: Chicken coops are rarely washed and can soil poultry carcasses with fecal bacteria that may make people sick. Our laboratory applied two commercially available products to experimentally contaminated coops. One product contained bleach, potassium hydroxide and a foaming agent. The other product contained vinegar and hydrogen peroxide and was mixed with a detergent. Both products were applied using a firefighting apparatus known as a compressed air foam system (CAFS). These materials were washed away using a garden hose or pressure washer as the treatments called for. Surface swabs were collected prior to and after each treatment to determine the reduction of bacteria on the surface, which would be an indicator of sanitation. We found that both treatments significantly made the surface cleaner when compared to water alone. The application of these products via a CAFS may be a practical and expedient way to clean and disinfect poultry cages.

Abstract: Transport coops are infrequently washed and have been demonstrated to cross-contaminate broiler carcasses. We hypothesized that peracetic acid or a chlorinated cleaner, commonly used within poultry processing plants, can also be used to disinfect transport coops when applied via a compressed air foam system (CAFS). A mixture of fresh layer manure and concentrated *Salmonella* Typhimurium (ST) was evenly applied to the floors of four pre-cleaned transport coops and allowed to dry for thirty minutes. Treatments consisted of a (1) water rinse only, (2) product application with a water rinse, (3) product application followed by power washing and (4) power washing followed by application of product. Each foaming treatment was applied with a compressed air foam system and allowed 10 min of contact time. Samples were aseptically collected from the transport coops prior to and following treatment using a sterile 2 × 2-inch stainless steel template and a gauze swab pre-enriched with buffered peptone water. The chlorinated cleaner significantly ($p < 0.05$) reduced aerobic bacteria and ST by 3.18 to 4.84 logs across application methods. The peroxyacetic acid (PAA) disinfectant significantly ($p < 0.05$) reduced aerobic bacteria and ST by 3.99 to 5.17 logs across application methods. These data indicate that a compressed air foam system may be used in combination with a commercially available cleaner or disinfectant to reduce aerobic bacteria and ST on the surfaces of commercial poultry transport coops.

Keywords: cleaning and disinfection; biosecurity; food safety; transportation coops; poultry

1. Introduction

Transportation coops have been shown to be a vector for cross-contamination during the 3–12 h transportation and holding period that occurs before birds are processed [1]. These coops contain organic matter and microorganisms left by previously transported flocks [2]. Salmonella and *Campylobacter* levels can increase by 20 to 40% during loading, transportation, and holding before being processed [3–5]. Transportation is a known stress factor in poultry production and is why studies show increasing levels of microorganisms during this event [6]. Poultry transportation coops are not required to be cleaned and disinfected prior to reuse, which may lead to cross-contamination between broiler flocks [5,7]. Broilers determined to be negative for *Campylobacter* become positive post-transportation in coops previously used for transport of *Campylobacter* positive flocks [7]. Research has been conducted to evaluate reductions in bacteria present on transportation coops by washing and allowing an extended drying time. These methods were found to be successful, but were considered impractical for the industry since this would require more coops and a large amount of space for drying [3].

Campylobacter and Salmonella are a concern within the industry because of their prevalence in poultry products [8]. Disinfectants such as peroxyacetic acid (PAA) are currently used in chillers at poultry processing plants because of its ability to reduce microorganisms, such as *Campylobacter* and Salmonella [9]. Guidelines to control and prevent these two microorganisms have been written and are in place for the poultry industry [10]. Researchers have collected carcass samples within poultry processing plants to determine where the highest loads of *Campylobacter* were found [11]. Mechanical feather removal within the processing plant is one area where bacterial load has been shown to increase, picker fingers cross-contaminate feather follicles with high levels of organic matter containing microorganisms which may further contaminate carcasses [12]. Lowering the number of microorganisms and organic matter entering the plant from transportation coops should result in less organic matter on carcasses and possibility reducing cross-contamination.

The poultry industry may use firefighting foam to depopulate birds during a reportable disease outbreak. The emergency technique has been conditionally approved by the American Veterinary Medical Association and the USDA-Animal Plant Health Inspection Service [13]. Foam is a quick alternative method to depopulate broilers that can be less labor intensive than gas asphyxiation [14]. Using a compressed air foam system (CAFS) may also be an efficient way to disinfect and sanitize poultry transportation coops. Disinfecting treatments using CAFS have been shown to reduce aerobic bacteria on layer cages and broiler transportation coops [15,16]. The food industry uses foaming disinfectants and cleaners to reduce microbial surface contamination, suggesting that a scalable approach using CAFS has potential.

In this study, we evaluated peracetic acid and a foaming cleaner that is commonly used by the poultry industry. Peracetic acid is a mixture of hydrogen peroxide and acetic acid. It is a robust disinfectant that can tolerate high organic loads yet decomposes into relatively safe by-products. It denatures proteins and increases cell wall permeability. The foaming cleaner was a proprietary formulation consisting of 5–10% potassium hydroxide, 1–3% sodium hypochlorite and a foaming agent. Alkaline ingredients are used to saponify lipids and help with the removal of organic matter. While the foaming cleaner was not labeled as a disinfectant, the strong base and sodium hypochlorite were expected to have some antimicrobial activity. Chlorine products are inexpensive and effective disinfectants that can kill or damage microbes due to oxidation of proteins and disruption of cell membranes. Unfortunately, they are also quickly depleted in the presence of organic matter [17].

The objective of the current study was to evaluate the disinfection of poultry transportation coops using a foam cleaner (FC), PAA + FA, or PAA + FA with a high-pressure water rinse (HPWR) prior to or following the foam application on aerobic bacteria and *Salmonella* recovery. A field study was conducted at a commercial poultry processing facility. This trial evaluated PAA + FA alone and with a HPWR prior to the foam application to evaluate aerobic bacteria present on poultry transportation coops. We hypothesized that the application of disinfectants or cleaners with foam using the CAFS would significantly reduce *Salmonella* and aerobic bacteria on broiler transport coops.

2. Materials and Methods

2.1. Experimental Design

Lab Trial 1–Peroxyacetic Acid with a High-Pressure Water Rinse - Aerobes and Salmonella Recovery. Lab trial 1 used four transportation coops, with each one representing a different treatment. Treatments consisted of a: (1) low-pressure water rinse (LPWR) only; (2) PAA + FA; (3) HPWR followed by the PAA + FA; and (4) PAA + FA followed by a HPWR.

Lab Trial 2–Foam Cleaner with a High-pressure Water Rinse–Aerobes and Salmonella Recovery. Lab trial 2 used four transportation coops, with each one representing a different treatment. Treatments consisted of: (1) LPWR; (2) FC; (3) HPWR followed by the FC; and (4) FC followed by a HPWR.

Field Trial—Peroxyacetic Acid with a High-Pressure Water Rinse–Aerobes. The field trial was conducted at a broiler processing facility and used three transportation coops. Treatments consisted of: (1) LPWR; (2) PAA + FA; and (3) a HPWR followed by PAA + FA.

The control for these studies was the LPWR, which involved the use of a standard garden hose to rinse each of the ten compartments of a transportation coop. A standard garden hose was moved from the left side to the right side of each compartment which took less than 30 s to perform the LPWR. Bricks were placed on one side of the coop to allow for drainage during each rinse. All treatments were given a 10 min contact time and followed by a LPWR of the transportation coops to remove any chemical residue. The concentrations that were used for all studies were the maximum concentrations recommended by the manufacturers. The HPWR used a (Briggs & Stratton, Milwaukee, WI, USA) power washer for 1 min at 3000 psi on each transportation coop.

2.2. Cleaners and Disinfectants

The FC that was used in specified lab trials was an alkaline/chlorine-based product (Chlor-A-Foam, Neogen Animal Safety, Lexington, KY, USA) and it was used at a 118.29 mL/L (4 oz/gal) concentration. This product contained its own foaming agent so a foam additive (FA) was not added to this product when used.

The PAA disinfectant (Peraside, Neogen Animal Safety, Lexington, KY, USA) that was used in specified trials was also used at a 118.29 mL/L (4 oz/gal) concentration. This product did not contain its own foaming agent, so a FA was added to this product when used. The FA (Perafoam, Enviro Tech Chemical Services, Inc., Modesto, CA, USA), was added at a 1% concentration.

2.3. Compressed Air Foam System (CAFS)

Foam is composed of air, soap, and water. We used a CAFS (Rowe CAFS LLC, Washington, AR, USA) that can produce 1874 L (495 gallons) of firefighting foam per minute. For each trial, 189.27 L (50 gallons) of tap water was measured into the tank of the CAFS followed by 5.92 L (200 oz) of FC or PAA with 1.89 L (64 oz) of the FA (PAA + FA). A 2.54 cm (1 inch) fire hose was used to apply the foam from the CAFS to the contaminated coops.

2.4. Transportation Coops

Four broiler chicken transportation coops (Bright Coop, Inc., Nacogdoches, TX, USA) were obtained from a local commercial broiler integrator for experimental purposes. Each coop represented an experimental unit/treatment and had ten holding compartments in a configuration of two columns with five rows. During experiments, ten pre-treatment and ten post-treatment samples were taken from each transportation coop.

The field study used three transportation coops containing market-age broilers that had recently defecated in the coops during transport to the processing plant and had probably never been cleaned.

2.5. Fecal Slurry

Feces were collected from single combed white Leghorn chickens housed at the Texas A&M University Poultry Research Center. Five hundred grams of organic matter, 500 mL of *Salmonella* Typhimurium (ST) and 500 mL of tap water were mixed. The ST was cultured in tryptic soy broth (Becton Dickinson and Company, Franklin Lakes, NJ, USA) for 24 h at 37 °C and passed every 8 h to be used to spike the fecal slurry before being blended and homogenized.

The final study, at the poultry processing facility, did not use the homogeneous fecal slurry method since the transport coops were recently contaminated by commercial broiler chickens.

2.6. Paint Roller Application

The homogenous fecal slurry was blended and placed in a paint roller tray and a clean paint roller was used to apply the slurry onto the entrance of each compartment at a width equivalent to the length of one roller (23 cm). The slurry applied onto the transportation coops was given a 30 min dry time to simulate industry conditions.

2.7. Bacterial Recovery/Sampling

Samples were taken from each of the ten compartments of each transportation coop after 30 min of drying time. The samples were collected using a sterile 5 by 5 cm gauze pad which was pre-soaked with 5 mL of buffered peptone water and stored in a 4 oz WHIRL-PAK® bag (NASCO, Fort Atkinson, WI, USA). A 5 by 5 cm stainless steel template was soaked in 100% ethanol and flame sterilized between samples. To avoid sampling overlap, all pre-treatment samples were taken from the left side of each compartment, and all post-treatment samples were taken from the right.

2.8. Culture

Samples were kept in 4 oz WHIRL-PAK® bags and homogenized by a stomacher blender (Seward Limited, Worthing, West Sussex, United Kingdom for 30 s at 265 rpm. A series of 10-fold dilutions were performed into Butterfield's dilution tubes, plated onto tryptic soy agar (Becton Dickinson and Company, Franklin Lakes, NJ, USA) and incubated for 24 h at 37 °C.

For lab trials 1 and 2 the addition of Xylose-Lysine-Tergitol 4 (XLT4; Becton Dickinson and Company, Franklin Lakes, NJ, USA) plates were used to evaluate Salmonella bacterial recovery and were plated from the same Butterfield's dilution tubes then incubated for 48 h at 37 °C. Sample WHIRL-PAK® bags were incubated for 24 h at 37 °C then 100 µL of each sample were transferred into corresponding Rappaport-Vassiliadis (RV) Salmonella enrichment broth(Becton Dickinson and Company, Franklin Lakes, NJ, USA). The RV tubes that were incubated for 24 h at 37 °C were then struck onto XLT4 plates and incubated for 24 h at 37 °C to determine how many positive samples were detected through selective enrichment.

2.9. Scanning Electron Microscopy

Transport coop flooring was cut into approximately 18 mm squares and then thoroughly washed and cleaned in 100% ethanol. Squares were then packaged and sterilized by ethylene oxide gas sterilization. Sterilized squares were individually placed in 50 mL conical centrifuge tubes containing 45 mL of tryptic soy both inoculated with 10 µL from an overnight culture of *Enterococcus faecalis* and incubated overnight at 37 °C on a horizontal shaker. After overnight incubation, squares were removed and preserved by emersion in 25 mL of a fixative containing 3% glutaraldehyde prepared in 50 mM phosphate buffer, pH 7.4, with 50 mM sucrose. After a 60 min incubation, squares were post-fixed in a solution of 1% osmium tetroxide in 100 mM phosphate buffer, pH 7.4, with 100 mM sucrose for an additional 60 min. Following osmication, squares were rinsed in distilled water, dehydrated in a graded ethanol series and critical point dried using CO_2. Squares were then mounted on aluminum stubs, sputter-coated with gold and examined using a scanning electron microscope (JEOL 6400, JEOL USA, INC, Peabody, MA, USA). Control squares were un-inoculated pieces of cage material that were placed on aluminum stubs and examined in the scanning electron microscope.

2.10. Statistical Analysis

Bacterial recovery data were subjected to a one-way ANOVA using the general linear model procedure (SAS Institute Inc., Cary, NC, USA), with means deemed significantly different at $p < 0.05$ and separated using Duncan's multiple range test.

3. Results and Discussion

The objective for lab trial 1 was to spike layer feces with *Salmonella* Typhimurium and evaluate whether a high-pressure water rinse (HPWR) step prior to or following the PAA + FA treatment would be an added benefit in reducing aerobic bacteria and Salmonella (Table 1). Transportation coops treated with PAA + FA alone or with a HPWR step prior to or following the treatment in both replications were statistically similar ($p < 0.05$) in reducing aerobic bacteria (4.10 to 5.17 logs) and Salmonella (3.99 to 4.58). The LPWR consistently had the lowest reductions in both replications when reducing aerobic bacteria (2.09 and 2.14 logs) and Salmonella (2.10 and 2.16 logs).

The objective for lab trial 2 was to spike the feces with *Salmonella* Typhimurium and evaluate whether a HPWR step prior to or following a FC would be an added benefit in reducing aerobic bacteria and Salmonella (Table 2). Treatments using a FC varied statistically in both replications. In replication 1, HPWR prior to the FC and the FC used alone had the greatest reductions and were statistically similar ($p < 0.05$) in reducing aerobic bacteria (4.05 and 4.23 logs, respectively). The FC followed by the HPWR was statistically different ($p < 0.05$) from all other treatments at 3.5 $\log_{10}$ reductions of aerobic bacteria and was greater than the LPWR. The LPWR had the lowest reduction of aerobic bacteria at 1.12 logs.

Table 1. Lab Trial 1: Reduction of aerobic bacteria and Salmonella on broiler transportation coops following a compressed air foam application of disinfectant and a high-pressure water rinse [1].

Treatment [2]	Rep1 Log_{10} Reductions Aerobic Plate Count [3,4]	Log_{10} Reductions Salmonella Plate Count	Direct Plating Incidence [5]	Selective Enrichment Incidence	Rep2 Log_{10} Reductions Aerobic Plate Count	Log_{10} Peductions Salmonella Plate Count	Direct Plating Incidence	Selective Enrichment Incidence
LPWR	$2.14^{*,b} \pm 0.47$	$2.10^{b} \pm 0.54$	10/10	10/10	$2.09^{b} \pm 0.29$	$2.16^{b} \pm 0.38$	10/10	10/10
PAA + FA Only	$4.71^{a} \pm 1.33$	$4.12^{a} \pm 0.26$	1/10	10/10	$4.77^{a} \pm 1.17$	$4.22^{a} \pm 0.41$	3/10	10/10
HPWR followed by PAA + FA	$4.10^{a} \pm 0.81$	$3.99^{a} \pm 0.65$	1/10	9/10	$5.17^{a} \pm 0.93$	$4.48^{a} \pm 1.03$	0/10	8/10
PAA + FA followed by HPWR	$4.42^{a} \pm 1.38$	$4.58^{a} \pm 1.21$	1/10	5/10	$4.89^{a} \pm 1.34$	$4.35^{a} \pm 1.35$	2/10	7/10

[1] All treatments were given a 10 min contact time and were followed by a LPWR of the transportation coops to remove any residual chemical. [2] LPWR = Low-pressure water rinse; PAA + FA = Peroxyacetic acid with a foam additive, and HPWR = High-pressure water rinse. [3] Values for reductions in aerobic bacteria and Salmonella recovery were calculated by subtracting post-treatment from pre-treatment samples. [4] Data are presented as mean ± SE, log_{10} reduction; $n = 10$ pooled samples per treatment; log reductions are subjected to a one-way ANOVA using the GLM procedure, with means deemed significantly different at $p < 0.05$ and separated using Duncan's multiple range test.[5] Salmonella incidence data is described as "X" out of 10 samples or 10/10 for 100% positive samples. * [a-b] Column values with different superscripts differ significantly ($p < 0.05$).

Table 2. Lab Trial 2: Reduction of aerobic bacteria and Salmonella on broiler transportation coops following a compressed air foam application of foam cleaner and a high-pressure water rinse [1].

Treatment [2]	Rep1 Log_{10} Reductions Aerobic Plate Count [3,4]	Log_{10} Reductions Salmonella Plate Count	Direct Plating Incidence [5]	Selective Enrichment Incidence	Rep 2 Log_{10} Reductions Aerobic Plate Count	Log_{10} Reductions Salmonella Plate Count	Direct Plating Incidence	Selective Enrichment Incidence
LPWR	$1.12^{*,c} \pm 0.39$	$1.82^{b} \pm 0.46$	10/10	10/10	$0.98^{d} \pm 0.51$	$0.65^{c} \pm 0.95$	10/10	10/10
FC Only	$4.05^{a} \pm 0.71$	$3.71^{a} \pm 0.59$	3/10	10/10	$3.59^{b} \pm 0.81$	$3.18^{b} \pm 0.85$	5/10	10/10
HPWR followed by FC	$4.23^{a} \pm 0.53$	$3.48^{a} \pm 0.54$	2/10	10/10	$4.84^{a} \pm 1.05$	$3.90^{a} \pm 0.33$	1/10	10/10
FC followed by HPWR	$3.50^{b} \pm 0.13$	$3.65^{a} \pm 0.13$	0/10	10/10	$2.78^{c} \pm 0.74$	$2.82^{b} \pm 0.72$	8/10	10/10

[1] All treatments were given a 10 min contact time and were followed by a LPWR of the transportation coops to remove any residual chemical. [2] LPWR = Low-pressure water rinse; FC = Foam cleaner; and HPWR = High-pressure water rinse. [3] Values for reductions in aerobic bacteria and Salmonella recovery were calculated by subtracting post-treatment from pre-treatment samples. [4] Data are presented as mean ± SE, log_{10} reduction; $n = 10$ pooled samples per treatment; log reductions are subjected to a one-way ANOVA using the GLM procedure, with means deemed significantly different at $p < 0.05$ and separated using Duncan's multiple range test.[5] Salmonella incidence data is described as "X" out of 10 samples or 10/10 for 100% positive samples. *, [a-b] Column values with different superscripts differ significantly ($p < 0.05$).

In the same lab trial *Salmonella* Typhimurium recovery was also evaluated and all three treatments using the FC were statistically similar ($p < 0.05$) to one another (3.17 to 3.65 logs). The LPWR had the lowest reduction at 1.82 logs of Salmonella and was statistically different than all other treatments. These data demonstrate that the FC is effective in reducing not only aerobes but Salmonella as well.

In replication 2 of lab trial 2 (Table 2) aerobic bacteria reductions for all coops were statistically different from one another. The greatest reduction was achieved from the HPWR followed by the FC, which was a 4.84 $\log_{10}$ reduction of aerobic bacteria. Another significant reduction came from the FC used alone with a reduction at 3.59 logs of aerobes. The FC followed by the HPWR with a reduction of 2.78 logs of aerobic bacteria also had a significant reduction. The lowest reduction was observed with the LPWR treatment at 0.98 log reduction of aerobic bacteria.

Replication 2 also evaluated the reductions of Salmonella. The authors found that the HPWR followed by the FC had the greatest statistically significant reduction of 3.90 logs of aerobic bacteria. The HPWR used prior to the use of the FC consistently proved to be the most effective way to reduce aerobic bacteria and Salmonella in both replications, which could be because the organic matter was removed prior to the product being applied. The organic matter that was used for lab trials had water added and *Salmonella* Typhimurium was blended to allow the slurry to be thicker in consistency and truer to organic matter that is naturally present on broiler transportation coops. According to Dvorak and Peterson 2009 [18] the removal of organic matter first is essential because it acts as a barrier to the microorganisms present and affects the efficacy of the disinfectant. They concluded that the efficacy of hypochlorites is rapidly reduced when a large amount of organic matter is present. Perhaps this is why we saw better results from the coops treated by the HPWR first followed by the disinfectant or cleaner in this lab trial. The FC used alone and followed by the HPWR statistically had similar reductions (2.82 and 3.18 logs). Finally, the LPWR statistically showed that it had the lowest reductions of Salmonella at 0.65 logs of aerobic bacteria.

The objective of the field trial was to evaluate whether PAA + FA alone or after a HPWR step would be effective in reducing aerobic bacteria on freshly contaminated broiler transportation coops from a poultry processing facility (Table 3). Similar results were seen in both replications. Significant reductions (1.72 and 2.32 logs, respectively) of aerobic bacteria were observed from coops treated with HPWR followed by PAA + FA in both replications. The HPWR proved to be effective in a field setting, which may be due to the removal of organic matter present that had not been washed previously.

Table 3. Field Trial—Reduction of aerobic on broiler transportation coops following a compressed air foam application of disinfectant and a high-pressure water rinse.[1.]

Treatment [2]	Replication 1 Log$_{10}$ Reductions Aerobic Plate Count	Replication 2 Log$_{10}$ Reductions Aerobic PlateCount
LPWR	$0.00^{*,c} \pm 0.66$	$0.42^{c} \pm 0.37$
PAA + FA	$0.88^{b} \pm 0.62$	$0.80^{b} \pm 0.34$
HPWR followed by PAA + FA	$1.72^{a} \pm 0.57$	$2.32^{a} \pm 0.40$

[1] All treatments were given a 10 min contact time and were followed by a LPWR of the transportation coops to remove any residual chemical. [2] LPWR = Low-pressure water rinse; PAA + FA = Peroxyacetic acid with a foam additive, and HPWR = High-pressure water rinse. [3] Values for reductions in aerobic bacteria were calculated by subtracting post-treatment from pre-treatment samples. [4] Data are presented as mean ± SE, $\log_{10}$ reduction; $n = 10$ pooled samples per treatment; log reductions are subjected to a one-way ANOVA using the GLM procedure, with means deemed significantly different at $p < 0.05$ and separated using Duncan's multiple range test. [*, a-b] Column values with different superscripts differ significantly ($p < 0.05$).

Researchers have suggested that high-pressure rinsing may be more effective to significantly reduce bacterial load than a LPWR and was what was seen in the results for our lab trials [3]. Their hypothesis to apply a HPWR proved to be effective in a field setting along with removal of organic matter which is what previous research and literature suggests. Stringfellow and co-workers [19] concluded that when using disinfectants, correct contact time, temperature, and amount of organic matter affects product efficacy. Furthermore, a multi-step protocol is required to effectively reduce the bacterial load found in cages [20]. The higher amount of organic matter seen in the present study led to the conclusion that the addition of a HPWR will further reduce bacterial load present on transportation coops. The PAA + FA used alone had a significant reduction of aerobic bacteria (0.88 and 0.80 logs). The LPWR had the lowest reduction concentrations (0.0 and 0.42 logs) for the field trials conducted.

Transport coop floors were power washed and treated with relatively high concentrations of disinfectant or cleaner during the lab and field trials. The researchers were surprised to continue to find bacteria present on surfaces that appeared to be smooth and clean. To further investigate this observation, a separate experiment was conducted within a microbiology lab. A coupon of broiler transport coop flooring was inoculated with *Enterococcus faecalis* and evaluated by a light and electron microscope (Figures 1–4). We found that the apparent smooth surfaces were actually scratched and covered in pits where bacteria could accumulate. It is possible that microbes may never encounter a cleaner or disinfectant due to the protection provided due to these imperfections.

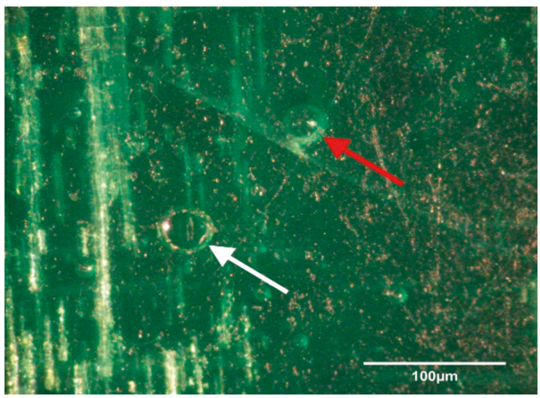

Figure 1. Light micrograph of uninoculated fiberglass flooring material depicting a hole (white arrow) in the material as well as subsurface air bubbles (red arrow). Sub-surface bubbles can be exposed to surface contamination as the surface wears with age.

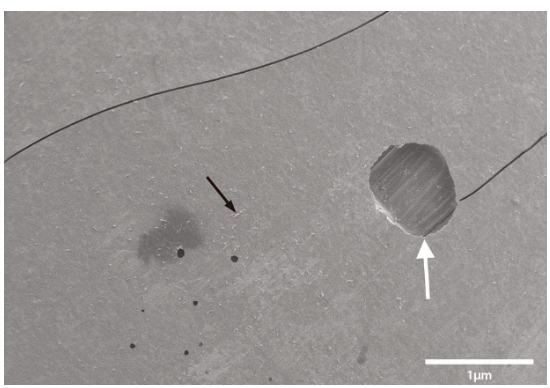

Figure 2. White arrow indicates hole in the surface of the fiberglass floor like that shown in Figure 1 (white arrow). Black arrow indicates bacteria colonizing the surface of the floor.

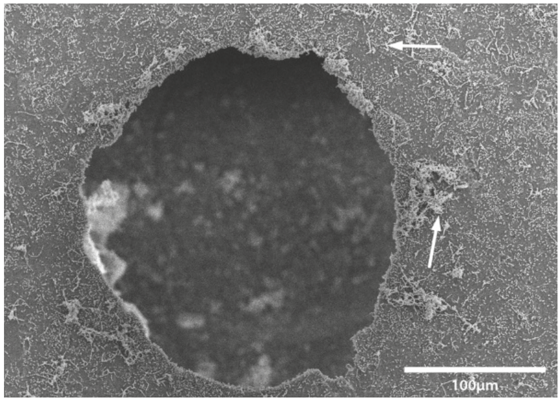

Figure 3. Higher magnification of a hole as seen in Figure 2 at 72 hours post inoculation with bacterial aggregates (white arrows).

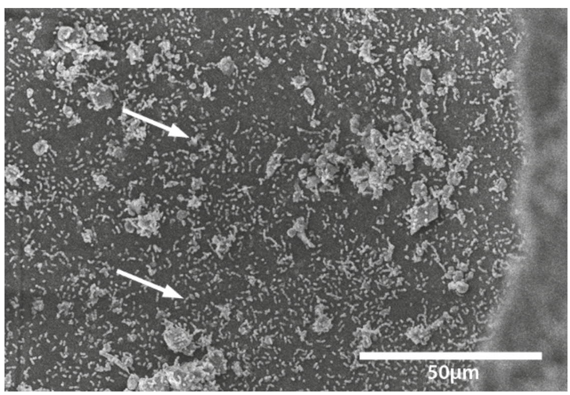

Figure 4. Micrograph of the bottom of the hole seen in Figure 3. Large aggregates of bacteria are evident adhering to the area of the hole (white arrows).Figures 2–4: Scanning electron micrographs of bacteria inoculated flooring at various timepoints after inoculation and magnifications.

The current study did not demonstrate whether the bacteria present were killed on the coops or were physically washed away but shows whether the bacteria were reduced or removed. As such, this observation may be irrelevant since the bacteria are no longer present on the transportation coops that can be a vehicle for cross-contamination. Continued research in a commercial setting may be needed. Furthermore, evaluations of bacterial counts on carcasses can be compared when taken from washed transport coops versus unwashed coops to further determine bacterial load. These products have already been approved by the Environmental Protection Agency, which means that they may be implemented in being used at a poultry processing facility. These data suggest that a CAFS application of cleaners and disinfectants may be used to significantly reduce Salmonella and aerobic bacteria on broiler transport coops. While a direct comparison was not made, coops from a commercial setting were found to be more difficult to clean and disinfect than coops which were contaminated in the laboratory.

4. Conclusions

A CAFS can be used to apply disinfectants with foam or foaming cleaners to effectively reduce aerobic bacteria which contaminate broiler transportation coops.

Common poultry disinfectants (peroxyacetic acid and chlorine releasing agents) when combined with a foam additive prove to be effective in reducing aerobic bacteria and Salmonella.

The addition of a HPWR used prior to or following the treatment did not improve efficacy in a laboratory setting but was beneficial in the field study.

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