



final report

Project code: W.LIV.0142

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PUBLISHED BY
Meat and Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Strategies to reduce inanition in sheep

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

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Abstract

Inappetence, the reduction in or lack of appetite with consequent reduced food intake (Blood and Studdert 1999), has been identified as a problem for sheep in the live export process, as a cause of death from inanition, and because of the association with the development of *Salmonella* outbreaks and subsequent death of animals. To characterise the problem with the typical consignments of sheep currently exported, sheep were monitored at a pre-embarkation feedlot in Western Australia using RFID tags and specially-designed tracking antenna to determine time spent at feed and water troughs. The patterns of attendance at feed and water troughs of 8,206 sheep from four export consignments were monitored for a range of 6 to 31 days. Inappetence was defined as sheep spending less time per day at the feed troughs than 2 standard deviations below the mean for the whole group, 28m 5s, and it took until day six in the feedlot for more than 95% of animals to be spending adequate time at the feed trough. Mortality of the monitored sheep was 0.85%, with enteritis (primarily salmonellosis)/inanition as the main cause of death. Other causes of death included pleuropneumonia and transit tetany. Statistical analyses highlighted difficulty in using feeding times to predict mortality at the feedlot.

A number of feeding strategies considered relatively easy for industry to implement were tested for the potential to increase the acceptance and consumption of a pelletised diet. A further 6048 sheep from 6 consignments were monitored in these tests. Feeding strategies tested at the feedlot did not apparently increase acceptance and consumption of the pellets. Housing sheep outside the raised sheds, with access to hay and/or pellets, for a day before entering the sheds, did not hasten feed acceptance or increase the number spending an acceptable period at the feed troughs, compared to those housed only in the shed. There was an additional risk of exposure to *Salmonella* in outside environments.

The addition of oats over the pellets did not result in any difference in uptake compared to sheep that were fed pellets alone. Spreading of chaff once per day over the pellets did alter the feeding pattern, and the sheep which were provided with chaff visited the feed troughs more often per day, but for shorter visits, than those given only pellets. In one experiment, provision of chaff did apparently hasten initial attraction to feed, with less sheep in the chaff group classed as inappetent on day 1, compared to those given only pellets.

The on farm provision of trail fed pellets for several preceding days, or the feeding of hay the day before trucking did not increase the time or number of visits at the feedlot feed troughs in the one tested group. Other on-farm factors, along with differences in transport conditions, handling, and prior medical and management practices may be influencing the adaptation to a pelleted diet at the feedlot. Further research is warranted to explore management practices that improve the resilience of sheep to novel conditions and feeds, resulting in consumption of a pellet diet at pre-embarkation feedlots in preparation for shipping.

Executive Summary

Inappetence, the reduction in or lack of appetite with consequent reduced food intake (Blood and Studdert 1999), has been identified as a problem for sheep in the live export process, as a cause of death from inanition, and because of the association with the development of salmonella outbreaks and subsequent death of animals. To characterise the problem with the typical consignments of sheep currently exported, sheep were monitored at a pre-embarkation feedlot in Western Australia using RFID tags and specially-designed tracking antenna to determine time spent at feed and water troughs. Between September 2011 and June 2012, a total of 8,206 sheep from four different consignments destined for live export were monitored electronically at feed and water troughs, for a range of 6 to 31 d.

Inappetence was defined as sheep spending less time at the feed troughs than 2 standard deviations below the mean daily time for the whole group (28m 5s). Based on this definition, it took until day 5 in the feedlot for less than 5% of animals to be spending inadequate time at the feed trough. Mortality of the monitored sheep was 0.85%. For the first six days, sheep that were alive at the end of their stay at the feedlot spent an average of 1h 12m 09s more per day at the feed troughs than sheep that died. There was no difference in time spent at water troughs (17m 45s \pm 14m 0s) between sheep that were alive at the end of the feedlot stay and sheep that died. Salmonella/Inanition was the leading cause of death (61.4 %) and occurred across all months. Transit tetany affected one group of Dorper ewes (0.36 %) in spring and pleuropneumonia was confirmed in (0.3 %) sheep in summer.

The time spent at the troughs per day by each individual, and their outcome as live or dead, was used in linear discriminant analysis to classify sheep as 'at-risk' or 'not-at-risk' based on a linear function incorporating previous days' feeding times. In the cases where 1, 2, 6, and 7 d have elapsed, only the previous day's feeding time was useful in predicting whether or not a sheep was at-risk. However, the number of misclassifications of sheep was large, with a high percentage of 'not-at-risk' sheep classified as 'at-risk'. Therefore, using this model to remove 'at-risk' sheep for different management would result in many additional sheep moved unnecessarily. This system for recording time at feed and water troughs has merit in identifying individual animals which are not eating, and could be used with automatic drafting systems or other non-intrusive management that remove such animals for different feed or attention.

There was some correlation between time spent at the feed troughs and entry, exit and change in BCS, but not sufficiently strong that recommendations could be made regarding an optimum BCS for the process. There was no indication of the previously reported issues whereby very fat sheep did not eat and developed a fatty liver/ketosis syndrome. Thus, it was not considered feasible or useful to remove the small number of sheep that were not eating from the large group for further study.

A number of feeding strategies considered relatively easy for industry to implement were tested for the potential to increase the acceptance and consumption of a pelletised diet. Between August 2013 and August 2014, 5,382 sheep from five different consignments destined for live export were monitored for a range of 4 to 22 days, with different feed or feeding strategies tested at the feedlot. In February 2015, 666 sheep destined for export from a farm in Southwest WA were selected for an on-farm preparation study.

Feeding strategies tested at the feedlot did not apparently increase acceptance and consumption of the pellets. Housing sheep outside the raised sheds, with access to hay and/or pellets, for a day before entering the sheds did not hasten feed acceptance or increase the number spending an acceptable period at the feed troughs, compared to those housed only in the shed. In one test of these protocols, sheep fed hay and pellets outside for one day before entering the shed had a much higher mortality, diagnosed as primarily due to salmonellosis, than the other groups. In a subsequent examination of the environments, more Salmonella organisms were detected in the outside environment, and in sheep exposed to that environment, than in the sheds and in sheep housed only in the shed, indicating the potential for environmental contamination. Provision of oats on top of the pellets did not increase the visits of time at the troughs compared to those supplied with pellets only. The addition of chaff did result in more visits to feed trough than when pellets alone were available.

The provision of trail fed pellets on farm did not increase the time or number of visits at the feedlot feed troughs. The tested group of sheep all rapidly accepted the pellets and spent more than the minimum time at the feed troughs. Other on-farm factors, along with differences in transport conditions, handling, and prior medical and management practices may be influencing the adaptation to a pelleted diet at the feedlot.

Further research is warranted to explore management practices that improve the resilience of sheep to novel conditions and feeds, resulting in consumption of a pellet diet at pre-embarkation feedlots in preparation for shipping.

Table of Contents

1	Background.....	6
2	Projective Objectives	9
3	Methodology	10
3.1	Electronic tracking system	10
3.1.1	Testing of the electronic system	11
3.2	Monitoring of sheep destined for export	11
3.2.1	Monitoring	11
3.2.2	Intervention	12
3.2.3	Preparation.....	14
3.2.4	Measurements & statistics.....	14
4	Results.....	15
4.1	Monitoring.....	15
4.1.1	Feed troughs	15
4.1.2	Water troughs	15
4.1.3	Body condition score	17
4.1.4	Mortality.....	19
4.1.5	Inappetence	21
4.1.6	Examples of individual sheep	23
4.2	Intervention.....	24
4.2.1	August 2013: Shed versus paddock with hay and/or pellets	24
4.2.2	December 2013: Shed versus paddock with hay and/or pellets.....	28
4.2.3	January 2014: Pellets and Oats	31
4.2.4	August 2014: Pellets and Chaff	33
4.2.5	October 2014: Pellets and Chaff	35
4.3	Preparation.....	37
4.3.1	February 2015: On farm pellets or hay.....	37
5	Discussion	40
6	Conclusions/Recommendations.....	45
7	Key Messages	47
8	Bibliography	50
9	Appendix.....	53
9.1	Statistical modelling to predict at-risk sheep	53

1 Background

Inappetence, defined as reduction in or lack of appetite with consequent reduced food intake (Blood and Studdert 1999), can affect up to 23% of sheep in feedlots (Norris *et al.*, 1990). Persistent inappetence leading to inanition, the end-stage condition resulting in death from prolonged reduction or cessation of feed intake, has been identified as a major cause of sheep mortality during the live export process (Richards *et al.* 1989). Whilst most inappetent sheep will commence feeding within 1 to 2 weeks of feedlotting (Bowman and Sowell 1997; Norris *et al.*, 1989), prolonged inappetence results in higher susceptibility to disease (Higgs *et al.*, 1993), such as salmonellosis. Sheep that do not eat at a pre embarkation feedlot are more likely to die during a voyage than those that do eat (Norris *et al.*, 1989; Norris *et al.*, 1990; Higgs *et al.*, 1996). Persistent inappetence and salmonellosis account for most deaths (over 60%) during the feedlot and shipping periods and there is a direct link between these illnesses (Richards *et al.*, 1989; Norris *et al.*, 1989; Norris *et al.*, 1990; Higgs *et al.*, 1991). Norris *et al.* (1989) found that sheep which failed to eat late in the feedlot period had 5.9 times greater risk of death due to salmonellosis than those that ate. Furthermore, Higgs *et al.* (1993) found death from salmonellosis exclusively in inappetent sheep. The detection, removal and remediation of inappetent sheep should occur early in the process, for better health and welfare outcomes (Foster and Overall, 2014). The detection of inappetent sheep has been trialled using marker dyes placed along the edge of feed troughs (Norris *et al.*, 1989; McDonald *et al.*, 1990) and also using real time observation. Both of these methods are time consuming and in the case of marker dyes, require frequent animal handling to record the marks. While real time observation does allow some degree of differentiation between individuals at particular time points, there is risk of error in identifying individuals and their activities in large groups.

This study aims to demonstrate the success of radio frequency identification (RFID) in identifying inappetent sheep. This technology can give a comprehensive description of feeding behaviour by recording frequency and duration of each individual at the feed and water trough. Previous methods of detecting inappetent sheep were unable to gather such a degree of information. A statistical model will be used to determine if the time sheep spend at a feed and water trough can be used to predict their risk of death while at a pre-embarkation feedlot.

Inanition within the live export industry was explored more than 20 years ago, prior to technology such as RFID being available. Those previous studies highlighted important issues to revisit, using technology that will allow larger scale work, and considering the types of sheep currently exported. Of interest is the incidence of inanition in specific lines of sheep. Previous studies found there is large variation between lines of sheep and proportion of inappetent animals in pre-embarkation feedlots (McDonald *et al.* 1990) and on ships (Norris *et al.* 1989a; Higgs *et al.* 1999), but the reasons are unclear. Higgs *et al.* (1991a) found that death rates during live shipment of hogget wethers were significantly lower than adult wethers. It was suggested that the differences in the basic appetite patterns of young and adult sheep may explain the lower mortality rate of the hogget wethers, but this was not explored (Higgs *et al.* 1991). Norris, *et al.* (1989b) were not able to define particular on-farm factors that were associated with the risk of inappetence. However, particular farms of origin were associated with increased mortality. There may be some farm management practices, or lines of sheep, that increase susceptibility to the stresses. Therefore those sheep are at greater risk of inappetence, development of disease such as salmonella, and therefore death. Given that

previous studies have shown variation in different lines of sheep, further investigation of specific lines could result in ability to isolate high risk lines of sheep and use preventative measure to reduce incidence of inanition.

Salmonella is considered more likely to cause clinical disease and death in animals that are not eating, most likely due to these sheep having lower resistance to enteric colonisation with salmonella organism. If sheep are not eating, rumen pH will increase and volatile fatty acid content will decrease. This creates a favourable environment for salmonellae to survive passage through the forestomachs (Radostits et al. 2000). Higgs, *et al.* (1993) found that persistently inappetent animals are significantly more likely to develop clinical salmonellosis and die during live export. However, no literature exists that investigates this assumption in a prospective manner; rather this conclusion is a result of retrospective analysis of mortality and cause of death data that sheep that did not eat in the feedlot were at a greater risk of dying during the voyage. Similarly, most of the recent diagnostic work on cause of death has been retrospective, such that sheep with no rumen fill at post mortem are considered to have died of inanition. While sheep that are not eating are thus considered more likely to develop salmonellosis, acute enteric salmonellosis will also decrease feed intake (Radostits, *et al.*, 2000), and the interaction between the two syndromes means that cause and effect, and therefore best practice in management, have been difficult to determine.

Two salmonella syndromes are considered to occur during the live export process, the acute enteric form that may result in rapid spread of disease and high mortality, and the persistent inappetence-salmonella – inanition complex (House, *et al.*, 2006). It may be important to distinguish between these manifestations for appropriate management, prevention or treatment. This study aims to investigate the impact of time in the pre embarkation feedlot on the incidence of inanition and clinical salmonellosis at the feedlot.

No current data exists for the incidence inappetence of sheep at the pre-embarkation feedlot or on the ship. However, a typical expectation within any intensive management system is that 2-5 % of sheep do not eat (Bell, *et al.*, 2003). Research conducted in pre-embarkation feedlots found the percentage of non-feeders in different groups of sheep ranged from 0.2 to 23 % (Norris, *et al.*, 1989a). The reason for this voluntary refusal to eat had not been determined. There are several possible scenarios and reasons. It is important for correct management and treatment to distinguish between animals that never eat and those that eat initially but then stop eating, and also to separate possible reasons for not eating from the metabolic consequences. This study aims to investigate relevant hormonal and physiological (including immunological) measures on inappetent and feeding sheep from the feedlot to determine if there are specific detectable differences between sheep that do and do not eat at the feedlot. It will also be determined whether sheep that are detected as inappetent at the feedlot can be treated or managed so that they regain their appetite, and whether they carry a high risk of becoming inappetent again if exposed to the same conditions in the feedlot.

The influence of the feeding regime on the development of inanition is unknown. Sheep entering a feedlot where the only feed is pellets, supplied in troughs, may not recognise that it is food, and therefore may not approach or eat. Timid sheep, or those that have little experience and therefore increased wariness of novel situations, may be fearful and never become confident to try the feed. Submissive or fearful animals may be intimidated or pushed aside by dominant, assertive animals, and not feed, although Norris, *et al.* (1990) suggested

from their work that sheep that die from inanition on ship are not inhibited from eating because of competition or social dominance. Chapple, *et al.* (1987) suggested that sheep initially had to overcome fear of the trough, then overcome the fear of the new feed and finally learn to prehend, chew and swallow grain. This pattern lasts less than two weeks in feedlot cattle (Hicks, *et al.*, 1990). In sheep, it has been found that the time taken for all sheep to begin consuming feed is generally two weeks (Chapple, *et al.*, 1987; Juwarini, *et al.*, 1981). However, for some sheep the period of inappetence lasts a lot longer and the shy feeding results in complications leading to death.

The background feed experiences of sheep entering the feedlots are varied, but no specific factors have been associated with increased risk of developing inappetence or dying (Norris, *et al.*, 1989b). While Norris, *et al.* (1989b) could not find any associations between prior supplementary feeding and feedlot feeding or shipboard mortality, the supplementary feeds given on farm were hay or grain (oats, barley, or lupins). There is no information whether those sheep that have prior experience with pellets are any less or more likely to become inappetent at the pre-embarkation feedlot. Norris, *et al.* (1989a) did find that early in the feedlot period, sheep held in sheds and fed only pellets had higher rates of inappetence than those held in paddocks and fed hay. By the end of the 5 to 7 day feedlot period in that study there were no significant differences in the number of non-feeders between the groups. However, provision of hay does not appear to be the best answer to preventing inappetence; rather it perhaps delays the adaptation to the pelleted diet. (Norris, *et al.*, 1989a) reported that there was a much higher percentage of sheep not eating pelleted feed at the time of loading if they had received hay in the feedlotting period, compared to sheep fed only pellets. This may indicate a preference of the sheep for hay, and unwillingness to try different feed if recognisable feed is available.

Management practices, such as how the feed is provided, can also influence whether sheep will eat. McDonald, *et al.* (1990) found that provision of centrally located feed troughs increased feed intake and the number of sheep eating, compared to locating the troughs on the fence lines. Once an animal starts eating a new feed, rumen adaptation takes two to three weeks, while rumen flora alter to produce a population that can safely use the new substrates. If, for instance, the feed ingested is high in soluble carbohydrates, there can be rapid overgrowth of acid producing rumen bacteria, such as *Streptococcus bovis* and *Lactobacillus* spp. The subsequent production of large quantities of lactic acid lead to rumen acidosis and digestive upsets and this may limit or completely stop further feeding. Shipper pellets are usually low in soluble carbohydrate, partly because under the current pre-embarkation requirements the time available for adaptation to new feed is short, and therefore such feed needs to be easily digested without adaptation problems. There is no information that there are adaptation problems with current feeding recommendations and feed.

This study aims to examine feeding management strategies that may reduce the incidence of inappetence including,

- on-farm backgrounding strategies that help sheep adjust from a pasture diet to a typical livestock export pelletised diet.
- feedlotting strategies that help sheep adjust from a pasture diet to a typical export pelletised diet at the pre embarkation feedlot.
- additives or other influences in the feedlot that increase uptake and consumption of a pelletised diet.

2 Projective Objectives

By February 2016 the Research Organisation will have:

STAGE ONE

1. Developed RFID technology which is capable of accurately identifying and tracking individual sheep at feed and water troughs at a pre-embarkation feedlot shed.

STAGE TWO

2. Determined the incidence of inanition in specific lines of sheep at pre embarkation feedlot.
3. Investigated the impact of time in the pre embarkation feedlot on the incidence of inanition and clinical salmonellosis at the feedlot.
4. Investigated relevant hormonal and physiological (including immunological) measures on inappetent and feeding sheep from the feedlot to determine if there are specific detectable differences between sheep that do and do not eat at the feedlot, and determine whether sheep that are detected as inappetent at the feedlot can be treated or managed so that they regain their appetite, and whether they carry a high risk of becoming inappetent again if exposed to the same conditions in the feedlot.

STAGE THREE

5. Investigated practically implementable on-farm backgrounding strategies that help sheep adjust from a pasture diet to a typical livestock export pelletised diet.
6. Investigated feedlotting strategies at the feedlot that help sheep adjust from a pasture diet to a typical export pelletised diet at the pre embarkation feedlot.
7. Investigated the use of additives or other influences in the feedlot that increase uptake and consumption of a pelletised diet.

STAGE FOUR

8. Produce a set of best practice guidelines for pre-embarkation treatment of sheep that aim to minimise the incidence of inanition/salmonella on the Australian sheep export industry
9. Provide recommendations for future work, based on the results of these investigations which further investigates and aims to reduce the stress of sheep when they enter the intensive situation at the pre-embarkation feedlot (and subsequently the export ship). Such work could form the basis of a subsequent project.

3 Methodology

All experimental procedures were reviewed and approved by the Murdoch University Animal Ethics Committee (Permit number R2598/13).

3.1 Electronic tracking system

ALEIS Pty Ltd (Capalaba, Australia) constructed tracking antennae using their patented technology. Twelve antennae were built into each trough to detect the RFID tag (134.2 Hz, half duplex, passive, uniquely numbered identification) on the sheep in close proximity. A transmit cycle for each antennae takes 80 ms (40 ms to transmit to the RFID tag and 40 ms to listen for a response) and only one antennae per panel transmits at any one time; therefore it take 960 ms for every antennae in the panel to transmit once. The settings for proximity and timing were chosen based on observations of sheep eating, such that a recorded incident was taken to indicate the presence of a sheep's head at the trough. The antennae detect a tag no more than 350 mm away, which corresponds to a distance of no more than 100 mm from the leading edge of the trough. Although this does not guarantee an animal in that position is eating, the system is designed particularly to detect animals that are not attending the troughs and, therefore, are not accessing feed.

The feedlot shed contained eight pens separated by metal fencing and gates. Tracking antennae were installed in four pens in one half of a sheep shed (Figure 1). Each pen was 10 x 25 m and had a mesh floor supported by wooden beams. The outer walls and the roof of the shed are constructed of corrugated metal. The outer walls are enclosed to a height of 0.7 m then are open to the roof (height of 2.5 m). There are six feed troughs and three water troughs in each pen, and pelletised feed and water is provided *ad libitum* through an automated system. For pens with tracking antennae, each trough was modified by the removal of the sloping metal wall of the trough above the area holding the feed, and antennae were installed with connections through to a central computer. The four pens fitted with antennae can hold up to 2,500 sheep under normal feedlot stocking rates.

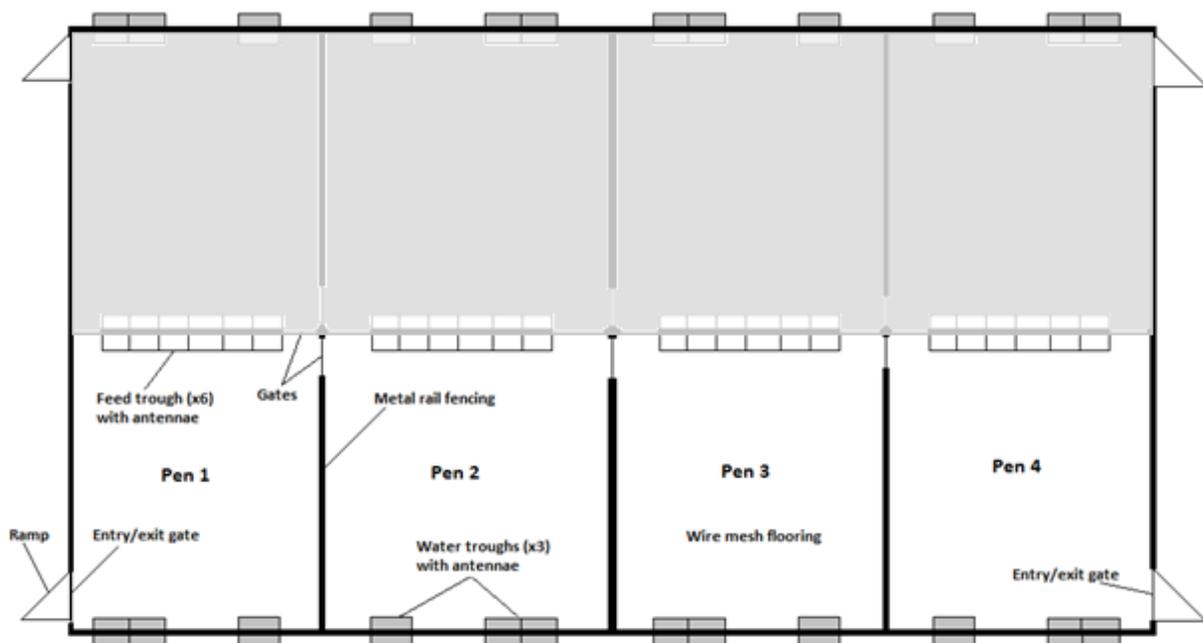


Figure 1. Schematic diagram of sheep shed showing location of feed and water troughs. Only the unshaded area was monitored.

3.1.1 Testing of the electronic system

Allflex National Livestock Identification System (NLIS) lightweight electronic reusable sheep tags were inserted into the right ear of 194 wethers on 24th June 2011. At 12 noon, these sheep were moved into pen 1 of the four pen system. Data was gathered successfully by the electronic system, and a live feed could be accessed remotely to confirm the system was operational. Data gathered was accessed through the database and was used to generate daily reports of sheep attending the feed and water troughs. .

After four days, gates were opened between all four pens and the tagged sheep moved into the other pens to mix with the neighbouring sheep. Data was gathered from all four pens to ensure the system in each pen was operational. For the duration of the feedlotting period, normal management procedures were followed, including removal of dead or moribund sheep, and addition of chaff to the feed troughs when feedlot staff detected sick animals.

The tags were removed from the sheep on 6th July 2011, and the sheep returned with the rest of that mob to continue normal management preparatory to shipping after approximately one week.

3.2 Monitoring of sheep destined for export

3.2.1 Monitoring

Between September 2011 and June 2012, 8,206 sheep from four consignments destined for live export were monitored for a range of 6 to 31 d. The sheep varied in breed (Merino, Dorper, Damara and crossbreeds of these), sex (primarily wethers, some rams and occasional ewes), age (lambs to 2 years old), and came from 284 farm origins, (Table 1). After being trucked to the feedlot from a variety of origins, sheep were either dealt with immediately, or after being held overnight in a yard off feed, depending on their arrival time. Sheep were identified with an Allflex NLIS electronic reusable RFID sheep tag placed in the right ear, weighed or scored for body condition (BCS), and then entered the monitoring pens with *ad libitum* access to pellets. One day before the sheep were due to exit the feedlot, the RFID tags were removed from the sheep, and sheep were scored for BCS. Normal feedlot management procedures were followed, including a daily check through the shed and the removal of dead or moribund sheep. Removed sheep were examined to diagnose illness or conduct a post-mortem to determine cause of death

Table 1. Details of all sheep monitored between September 2011 and June 2012.

Pen	Breed	Number of sheep	Days monitored	Number of different farm origins
September 2011				
1	Merino & Merino x Damara wethers	504	31	82
2	Merino wethers	503	26	80
3	Dorper ewe lambs	738	16	1
4	Dorper ram lambs	711	16	1

January 2012				
1 & 2	Merino wethers	1180	17	3
3 & 4	Merino	1134	15	11
February 2012				
2	Merino wethers	607	22	4
3	Merino Rams & wethers	357	7	2
June 2012				
1	Merino wethers	634	7	35
2	Merino wethers	566	7	31
3	Merino wethers	736	7	35
4	Merino wethers	536	6	94

3.2.2 Intervention

Between August 2013 and August 2014, subsets of sheep from five consignments destined for live export, totalling 5,382 Merino sheep (primarily wethers, some rams and occasional ewes), aged lambs to 2 years old were monitored for a range of 4 to 22 days. Sheep were trucked to the feedlot, unloaded and dealt with immediately. All sheep were then identified with an Allflex NLIS electronic reusable RFID sheep tag placed in the right ear, and scored for BCS. One day before the sheep were due to exit the feedlot, the RFID tags were removed from the sheep, and sheep were scored for BCS. Normal feedlot management procedures were followed, including a daily check through the shed and the removal of dead or moribund sheep. Removed sheep were examined to diagnose illness or conduct a post-mortem to determine cause of death

Shed vs. Paddock trials

Sheep were randomly allocated to three treatment groups; with one group housed in the monitoring pens, and two groups housed in separate outdoor paddocks overnight (Table 2). This took place twice; in August 2013 and December 2013, with different groups of sheep. Sheep housed in the monitoring pen had access to pellets only. One paddock housed group had access to pasture and pellets overnight, whilst the other paddock housed group had access to pasture, hay and pellets overnight. The following day, all paddock housed sheep were moved to the monitoring shed and housed there for the remainder of the trial, with *ad libitum* access to pellets only.

Table 2. Details of all sheep monitored for shed vs. paddock trials.

Treatment	Number of sheep
August 2013 (monitored for 6 days)	
Shed/pellets	161
Paddock/pellets	161
Paddock/hay/pellets	162
December 2013 (monitored for 24 days)	
Shed/pellets	195
Paddock/pellets	210
Paddock/hay/pellets	207

Supplementary feed trials

This trial took place in the monitoring pens where sheep had *ad libitum* access to pellets. The sheep used in this trial were a mix of merino wethers, ewes and rams. In January 2014, sheep were randomly allocated to two treatments groups; pellets only or pellets and oats. In August and October 2014, sheep were randomly allocated to two treatments groups; pellets only or pellets and chaff. In treatments where chaff or oats were used, approximately 20 L was poured over the pellets evenly in each feed trough.

Table 3. Details of all sheep monitored for supplementary feed trials.

Pen	Number of sheep	Days monitored
January 2014		
Pellets	717	4
Oats & pellets	744	
August 2014		
Pellets	1023	6
Chaff & pellets	981	
October 2014		
Pellets	411	8
Chaff & pellets	410	

3.2.3 Preparation

In February 2015, 666 sheep destined for export from a farm in Southwest WA were selected for an on-farm preparation study. All sheep were identified with an Allflex NLIS electronic reusable RFID sheep tag placed in the right ear, and scored for BCS. Sheep were randomly allocated to one of three groups: access to pellets (trail fed in the paddock) for three days before departure to feedlot, access to hay (in the paddock) one day before departure, and a control group which did not receive any supplementary feed. All sheep followed standard protocol of feed and water withdrawal overnight before being trucked to the feedlot. Once at the feedlot sheep were housed in the monitoring shed with *ad libitum* access to pellets. One day before the sheep were due to exit the feedlot, the RFID tags were removed from the sheep and they were scored for BCS. Normal feedlot management procedures were followed, including a daily check through the shed and the removal of dead or moribund sheep. Removed sheep were examined to diagnose illness or conduct a post-mortem to determine cause of death.

3.2.4 Measurements & statistics

Each time an antenna transmitted and detected an animal tag the data was stored. This data was collated to create specific reports using software written for the tracking system. Using this software, the time each individual attended the troughs ($h \cdot d^{-1}$) and the number of visits to the troughs can be accessed for any time period. Descriptive data was generated, including average time per day spent at the troughs, number of sheep attending the troughs for varying periods of time, and cumulative time spent at the troughs.

Differences in time at feed and water troughs for sheep alive and dead at the end of the feedlot period, and between shipments, was analysed using ANOVA (StatSoft Inc, Tulsa, OK, USA). Difference between breeds could not be compared between shipments because not all breeds were present in all shipments. Correlations between body condition score and total time and average time per day spent at the feed troughs was analysed using Spearman's Rank Correlation.

The time spent at the troughs per day by each individual, and their outcome as live or dead, was used in linear discriminant analysis to classify sheep as 'at-risk' or 'not-at-risk' based on a linear function incorporating previous days' feeding times. The statistical modelling to predict at – risk sheep is described in more detail in the Appendix.

For the intervention and preparation trials, comparisons between treatments was analysed using Repeated Measures ANOVA, and comparisons of the number of sheep considered at-risk and those that weren't was performed using Kruskal-Wallis ANOVA.

Standard deviation from the mean was calculated by calculating total time at feed troughs for each sheep and dividing by their individual length of stay at the feedlot (i.e. if they died their duration was shorter than an animal that was alive when the ear tag was removed) to produce an average time at feed troughs for each sheep. The mean and standard deviation was then calculated.

4 Results

4.1 Monitoring

The system operated successfully by providing daily data on all consignments of sheep and recording dead or removed sheep as not accessing the troughs. Of the RFID tags used in this study, 99.8% were still in situ in the ear at exit, and still functioning at that time, as detected by the portable wand. Of the tags that did not operate correctly, 13 did not record those sheep approaching the feed or water troughs for the time they were at the feedlot (7 to 17 days) and one tag recorded the sheep being at the feed trough almost 3 times longer than the average during the first 2 day, but did not record any data at the water troughs. Of these 14 sheep, nine were recorded at exit, indicating that these tags were working. The fate of the five sheep not recorded at exit is unknown, and these sheep may have been mixed with another pen of sheep adjacent to the experimental pens.

4.1.1 Feed troughs

Sheep spent an average of 1h 35m 41s \pm 33m 48s at the feed troughs per day. In a comparison between sheep that remained alive and sheep that died during the feedlot period (Table 4; Fig. 2) it was found that sheep that remained alive:

- spent on average 1h 12m 30s longer per day at the feed troughs (corrected for day of death) ($p>0.001$).
- visited the feed troughs more often (corrected for day of death) ($p>0.001$).
- spent longer at the feed troughs per visit ($p<0.001$)

4.1.2 Water troughs

Sheep spent an average of 17m 45s \pm 14m 4s at the water troughs per day (mean for all sheep, Table 4). More time was spent at the water troughs in January compared to the other shipments ($p>0.001$) by both sheep that remained alive and those that died during the feedlot period (Table 5; Fig. 3). The number of visits to the water troughs per day was not different between sheep alive or dead at the end of the feedlot period or between shipments.

Table 4. Mean time (h:mm:ss) and number of visits per day at feed and water troughs for sheep that remained alive and/or died during feedlot period.

	All sheep	Alive	Died
Feed troughs			
Average time per day	1:35:41 \pm 0:33:48	1:36:22 \pm 00:33:09	0:23:39 \pm 0:24:26
Average number of visits per day	33.6 \pm 12.54	33.8 \pm 12.33	8.4 \pm 8.92
Average time per visit	0:02:58 \pm 0:00:53	0:02:58 \pm 0:00:53	0:02:36 \pm 0:01:17

Water troughs

Average time per day	0:17:54 ± 0:14:04	0:17:02 ± 0:13:16	0:31:39 ± 0:29:44
Average number of visits per day	13.5 ± 6.68	12.9 ± 6.42	13.2 ± 7.70
Average time per visit	0:01:15 ± 0:00:35	0:01:15 ± 0:00:34	0:02:11 ± 0:01:15

Table 5. Average time per day spent at water troughs by sheep that remained alive or died during the feedlot period in each consignment (h:mm:ss). Significant differences are indicated ($p < 0.01$).

	Alive	Died
September 2011	0:16:12 ± 0:10:45 ^a	0:21:34 ± 0:17:06 ^a
January 2012	0:23:48 ± 0:16:00 ^b	0:40:46 ± 0:34:28 ^b
February 2012	0:14:35 ± 0:10:12 ^a	0:16:07 ± 0:11:08 ^a
June 2012	0:12:37 ± 0:11:12 ^a	0:12:37 ± 0:13:02 ^a

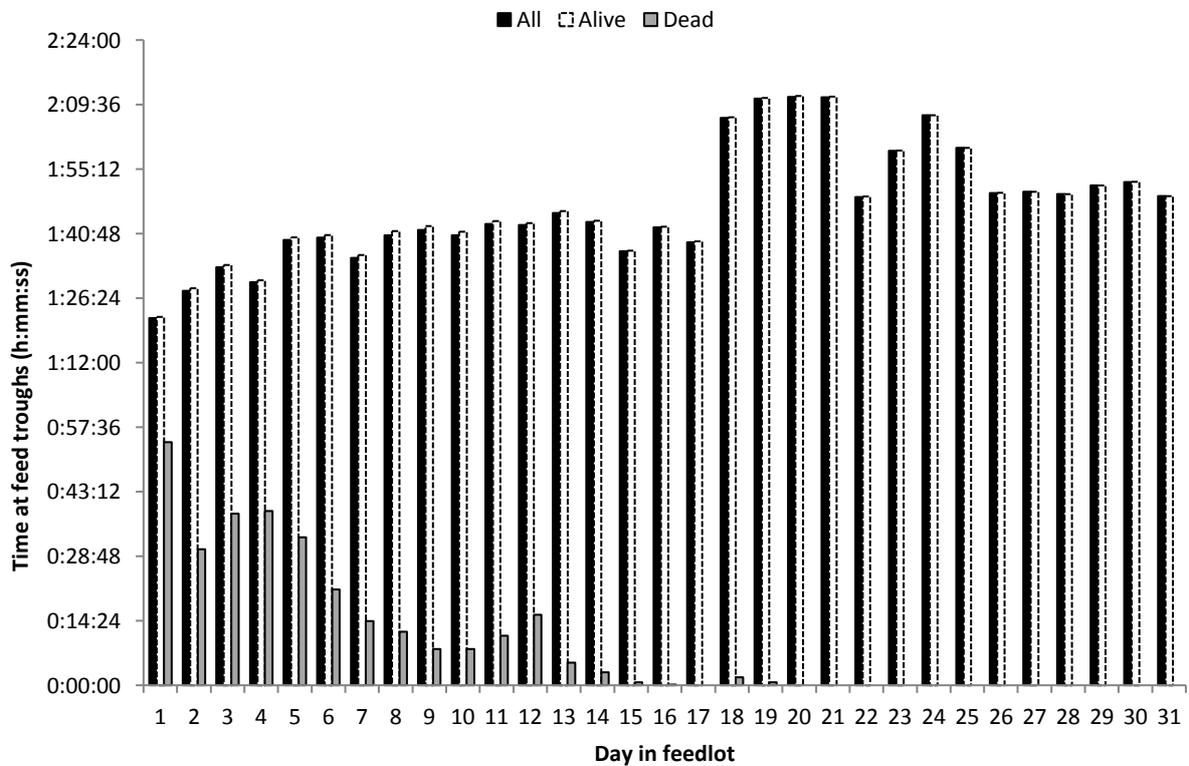


Figure 2. Average time per day spent at feed troughs by all sheep and those that remained alive or died during the feedlot period (h:mm:ss).

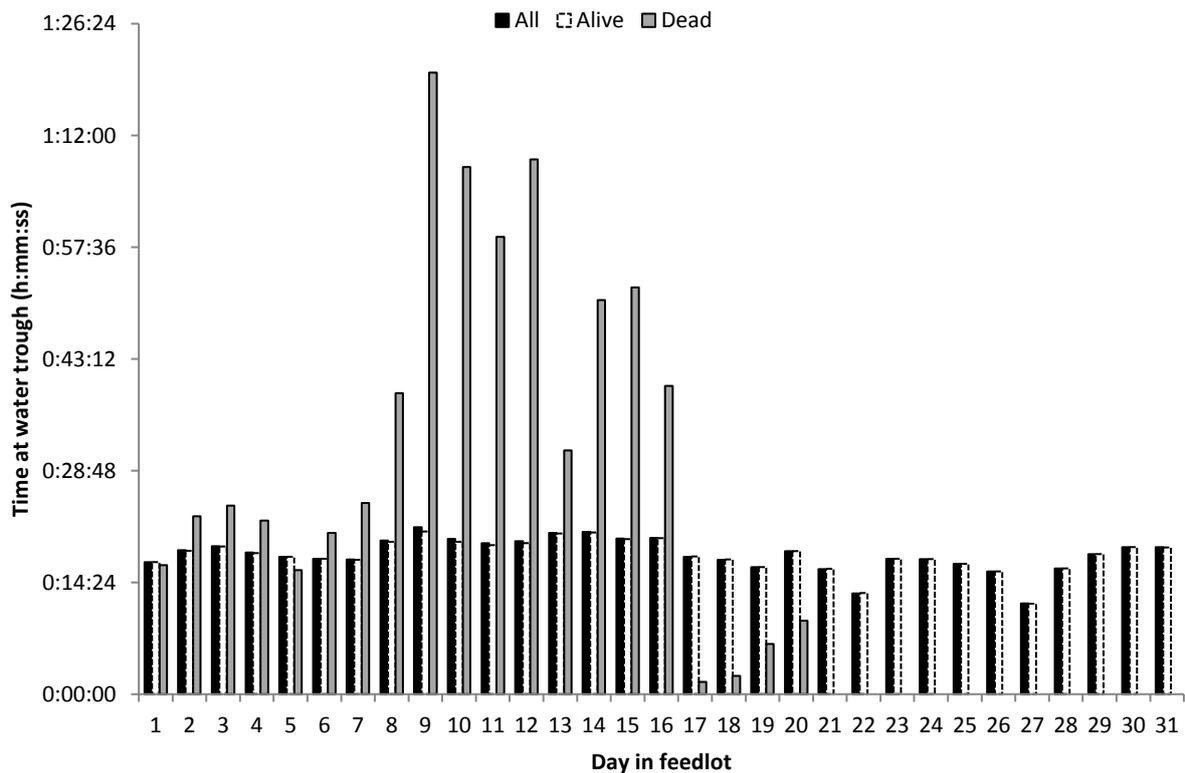


Figure 3. Average time per day spent at water troughs by all sheep and those that remained alive or died during the feedlot period (h:mm:ss). *Increases in time at water troughs observed on days 8 to 16 in sheep that died are from the sheep in the January 2011 shipment.*

4.1.3 Body condition score

On entering the feedlot, the BCS of sheep ranged from 0.5 to 3.5 (mean 1.6 ± 0.45). On exiting the feedlot, the BCS ranged from 0.25 to 4.0 (mean 2.4 ± 0.53). There were significant correlations between entry and exit BCS and average time per day and total time spent at the feed troughs ($p < 0.05$). There was also a significant correlation between BCS change and total time at feed troughs ($p < 0.05$) (Fig. 5). There were significant correlations between entry, exit and BCS change with average time per day and total time at the water troughs ($p < 0.05$) (Fig. 6). Sheep that had low entry, exit and changes in BCS tended to spend less time at the feed and water troughs.

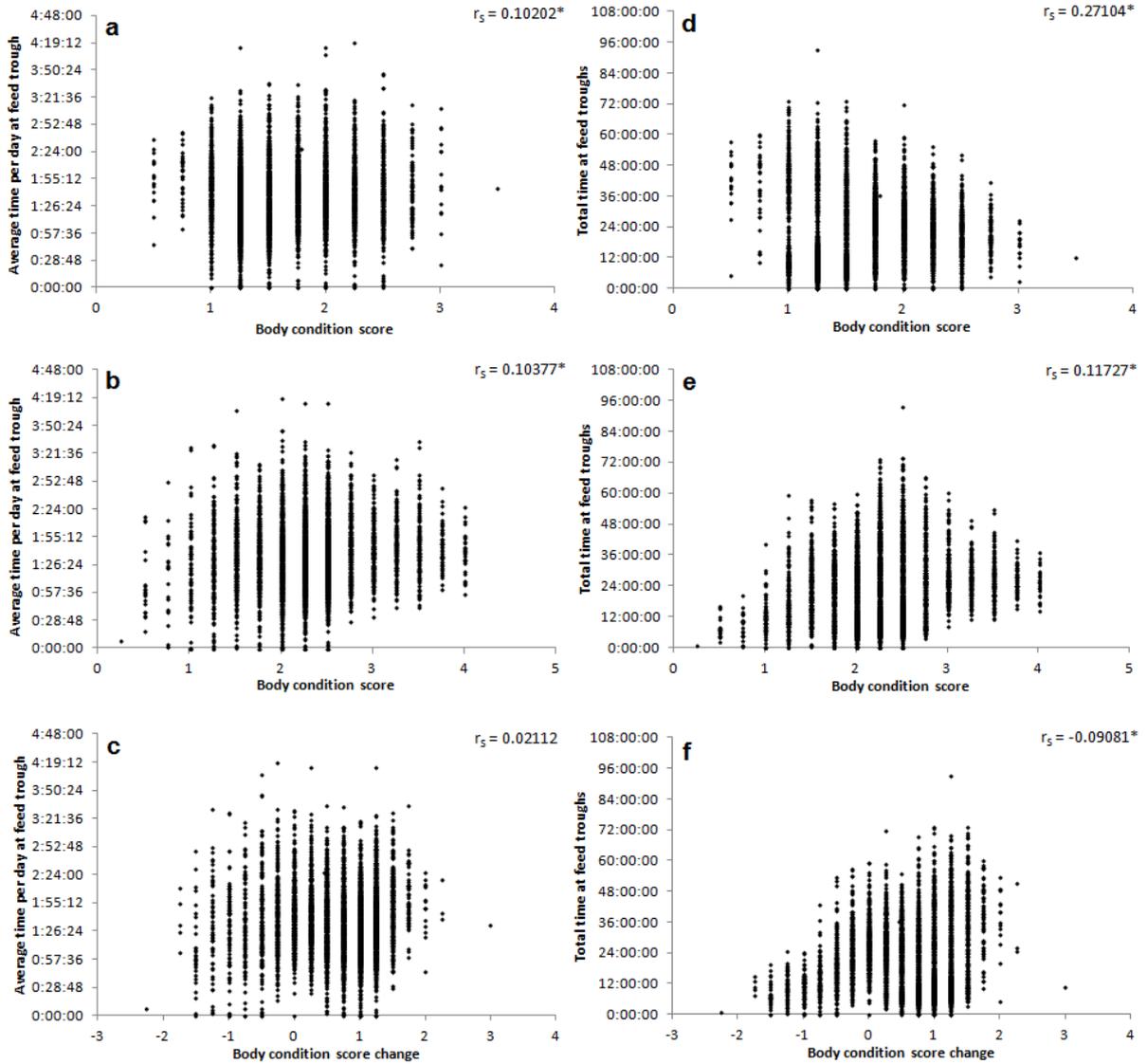


Figure 5. Correlations of average time (h:mm:ss) per day spent at the feed troughs with entry BCS (a) exit BCS (b) and change in BCS (c), and total time spent at the feed troughs throughout the feedlot period with entry BCS (d) exit BCS (e) and change in BCS (f). *Significant Spearman Rank Correlations are indicated.

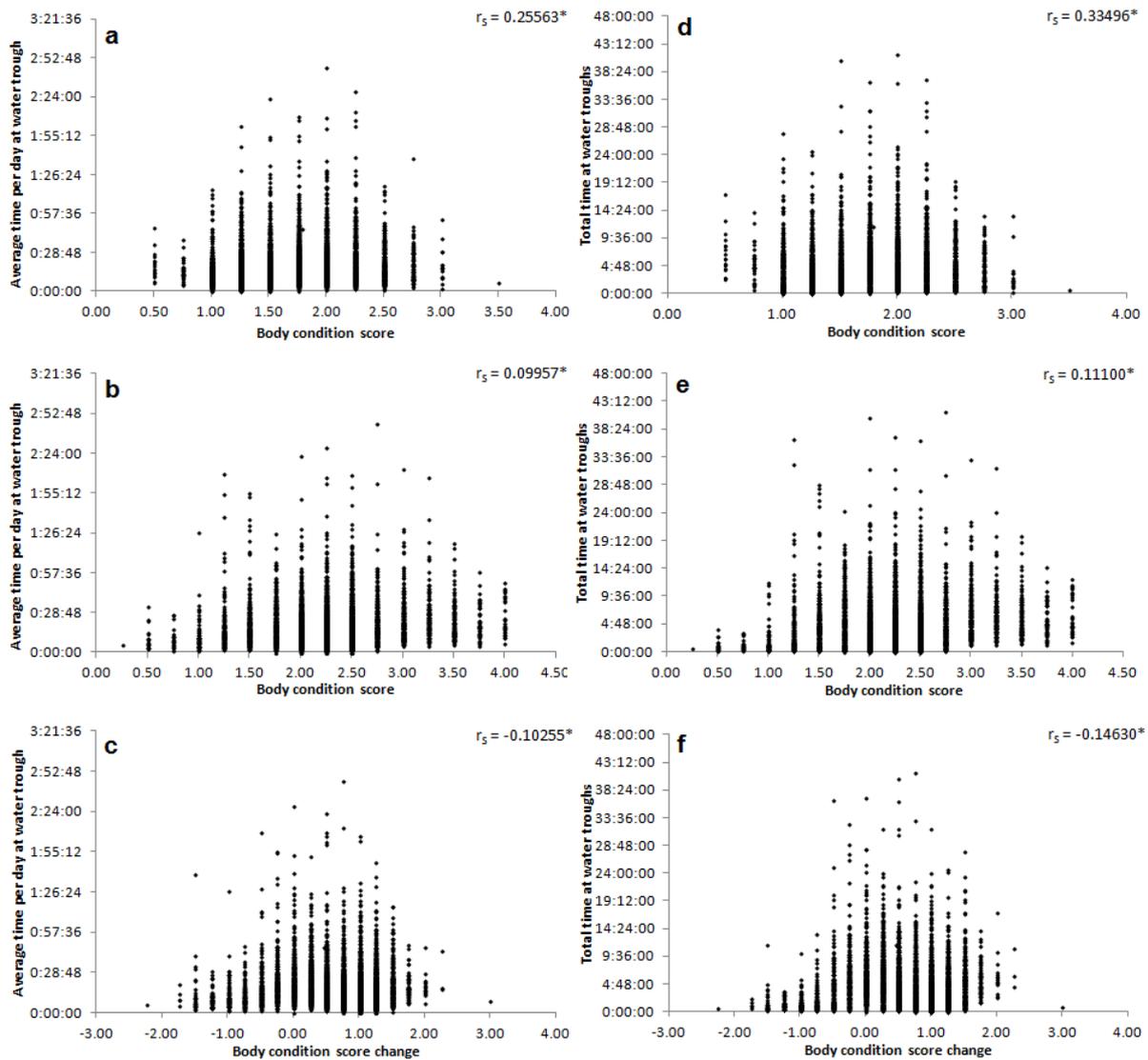


Figure 6. Correlations of average time per day (h:mm:ss) spent at the water troughs with entry BCS (a) exit BCS (b) and change in BCS (c), and total time spent at the water troughs throughout the feedlot period with entry BCS (d) exit BCS (e) and change in BCS (f). *Significant Spearman Rank Correlations are indicated

4.1.4 Mortality

Of the sheep monitored, 0.85% died, with the highest death rate in January 2012 (Table 6). Salmonella/inanition was the leading cause of death (61.4%) and was present across all months. The majority of sheep that died from salmonella/inanition were classified as inappetent, having spent less than 28m 5s (-2 SD below the average) at the feed troughs per day (Fig. 7). There was no such relationship with time at water troughs (Fig. 8). Hypocalcaemia/transit tetany affected one group of Dorper ewe weaners in September 2011, with animals having tetanic spasms before dying. Blood samples from affected animals were analysed as having low blood calcium concentrations and they were successfully treated with subcutaneous calcium borogluconate (Calcigol®). Pleuropneumonia as a primary cause of death was reported in 7 sheep in January 2012, and was also present to some extent in the sheep from that group diagnosed with salmonellosis as the primary cause of death.

Table 6. Cause of death, and number of sheep dead in each pen.

	September 2011	January 2012	February 2012	June 2012
Salmonella	9 (0.36%)	27 (1.17%)	4 (0.41%)	3 (0.12%)
Transit tetany	9 (0.36%)			
Shearing trauma	4 (0.16%)			
Mouth fistula	1 (0.04%)			
Bloat	1 (0.04%)			
Pneumonia		7 (0.30%)		
Enterotoxaemia (Etox: Clostridium perfringens infection)		1 (0.04%)		
High worm egg count			1 (0.10%)	
Unknown		3 (0.13%)		

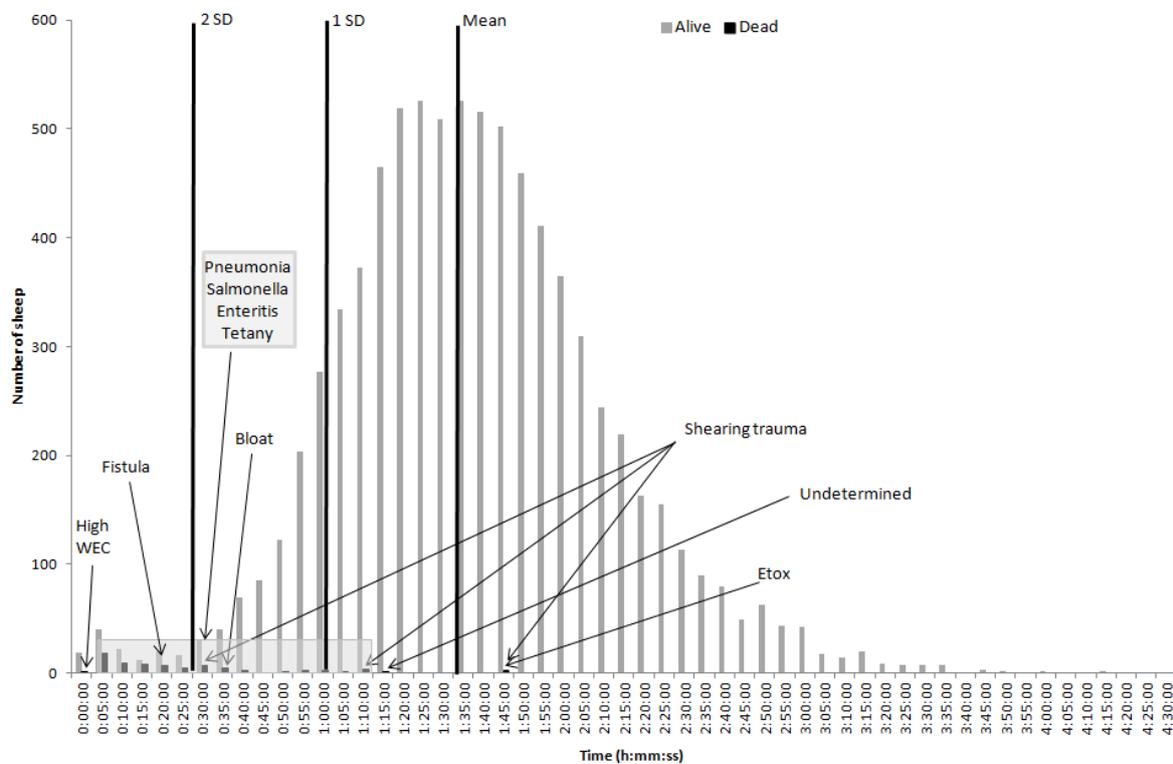


Figure 7. Histogram of the average daily time spent at the feed troughs. Sheep that died are represented by dark bars, and cause of death is indicated. The light grey box indicates

the range of sheep that died from pneumonia, salmonellosis, enteritis and transit tetany. The mean daily average, -1SD and -2SD below the mean are shown.

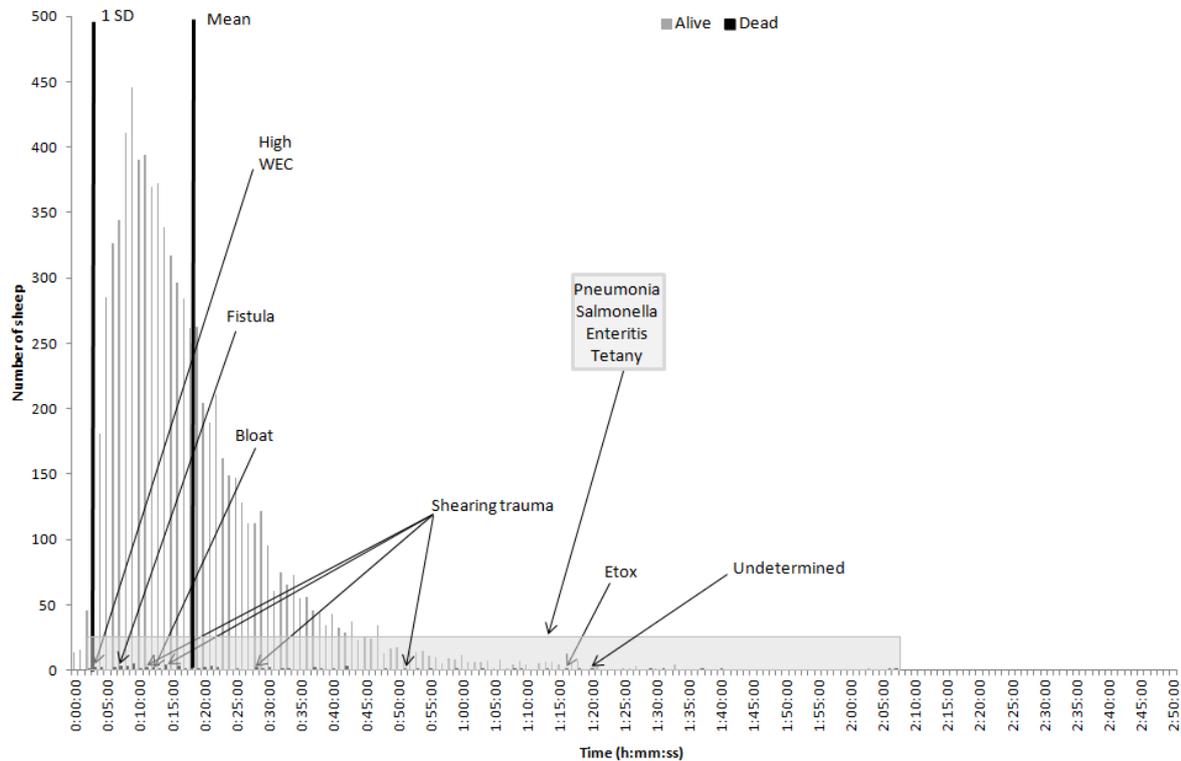


Figure 8. Histogram of the daily average time spent at the water troughs. Sheep that died are represented by dark bars, and highlighted causes of death are indicated. The light grey box indicates the range of sheep that died from pneumonia, Salmonella, enteritis and transit tetany. The mean daily average and -1SD below the mean are shown.

4.1.5 Inappetence

The distribution of daily average time at the feed troughs is shown in Figure 7, where the mean, and one (1h 01m 17s) and two standard deviations (SD) (28m 5s) below the mean are highlighted. The majority of animals which died spent an average daily time at the feedlots which was shorter than the mean minus one SD, while the average time per day spent at the feed troughs by sheep which died (corrected for day of death) was 23 m 33 s \pm 24m 11s, for the range of numbers of days monitored.

It was not possible, due to the low mortality rate, the range of days for which different groups were monitored, and the variable patterns of feeding, to effectively calculate the minimum daily time at the feed trough that was directly related to a higher risk of mortality (Appendix 1). Therefore, based on observations of the sheep including change in BCS, the pattern of feeding, and mortality, inadequate feeding time, or inappetence, was defined as sheep spending less time per day at the feed troughs than 2 standard deviations below the mean for the whole group, 28m 5 s. Figure 9 illustrates the percentage of sheep attending the feed troughs each day that were either more or less than -2 SD from the mean. The daily variation in time at feed troughs plateaued by day 6. Sheep that remained alive increased their time

spent at the feed trough over the first 6 days, whereas sheep that died decreased their time at the feed troughs. Time at water troughs was consistent for both sheep that were alive on exit from the feedlot and for those that died during feedlotting, and this did not vary greatly between days (Fig. 10).

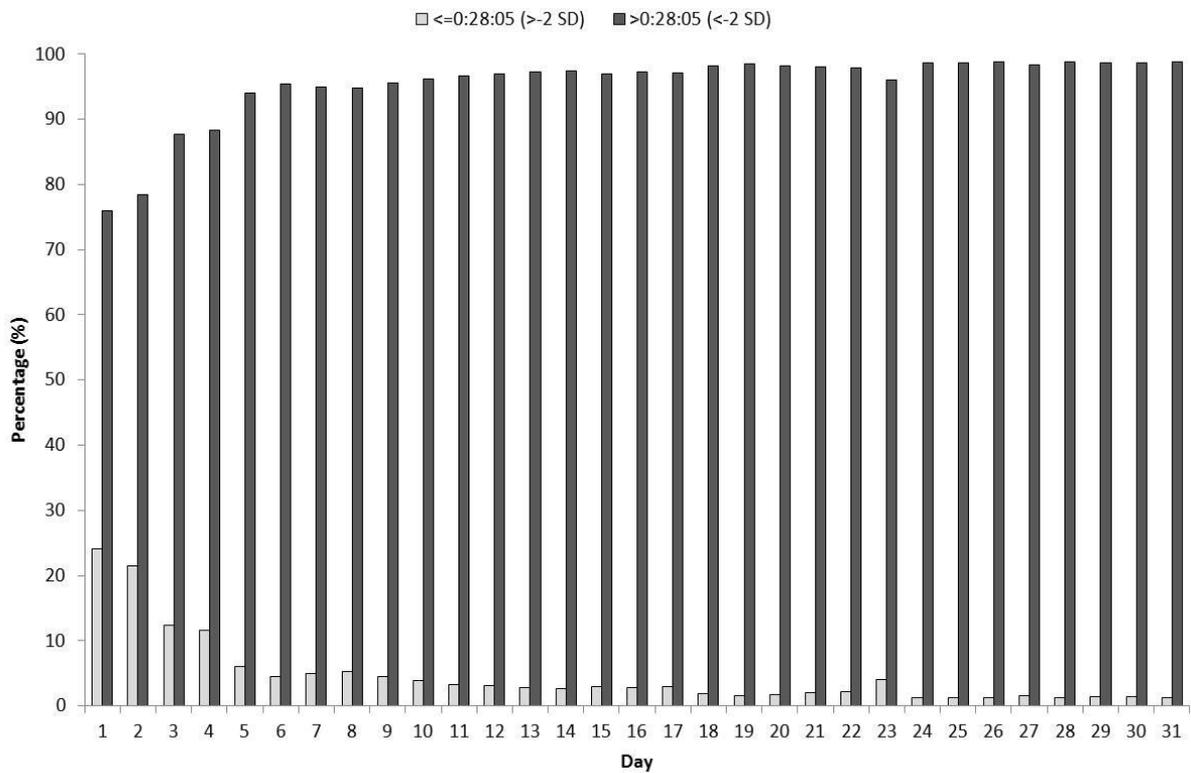


Figure 9. Percentage of sheep attending the feed troughs for less than or more than -2 SD from the mean (-2 SD = 28m 5s). Dorper ewes removed from days 6 and 7 data set due to absence for shearing.

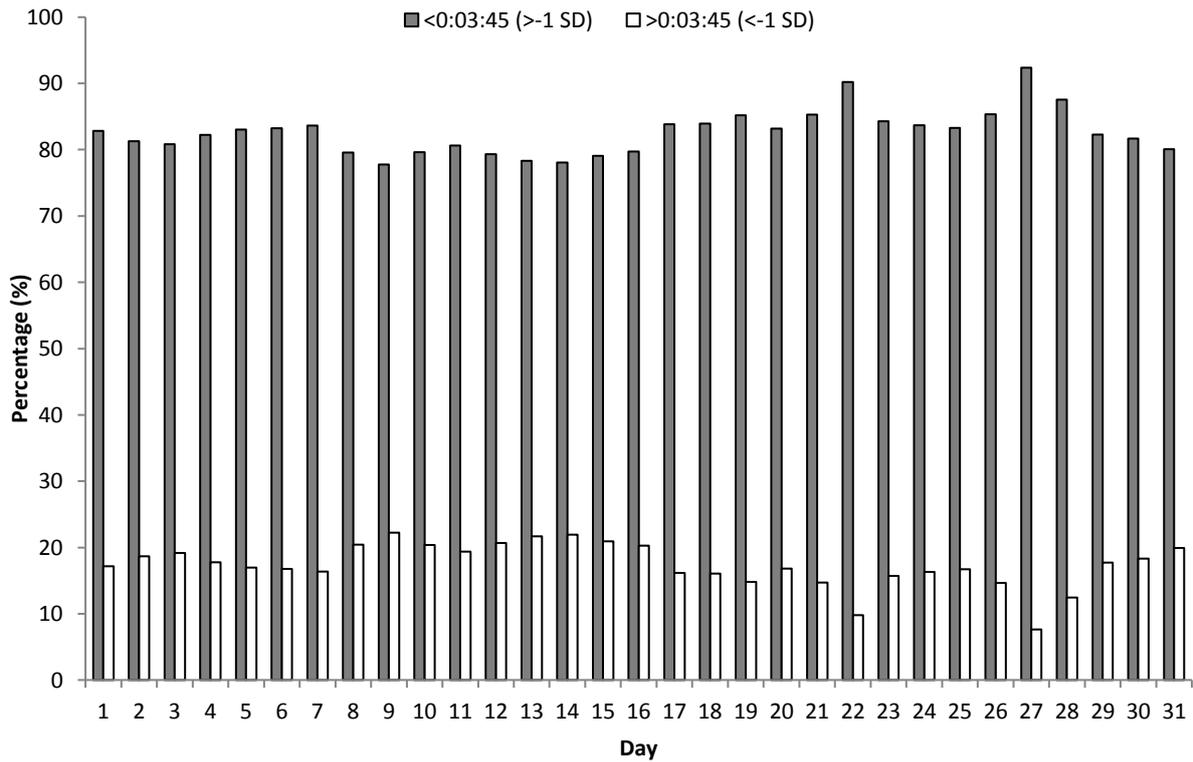


Figure 10. Percentage of sheep attending the water troughs for less than or more than -1 SD from the mean (-1 SD = 3m 45s). Dorper ewes removed from days 6 and 7 data set due to absence for shearing.

4.1.6 Examples of individual sheep

The pattern of time at feed troughs varied for sheep that died (Fig. 11). Some sheep spent a substantial amount of time at the feed troughs immediately on entry into the feedlot, and gradually went off their feed. Other sheep spent some time at the feed troughs on entry to the feedlot, spent none or little time at feed the next day, then returned to the feed troughs the day after, continuing with this intermittent feeding pattern until they eventually were recorded as not attending the feed troughs at all and died.

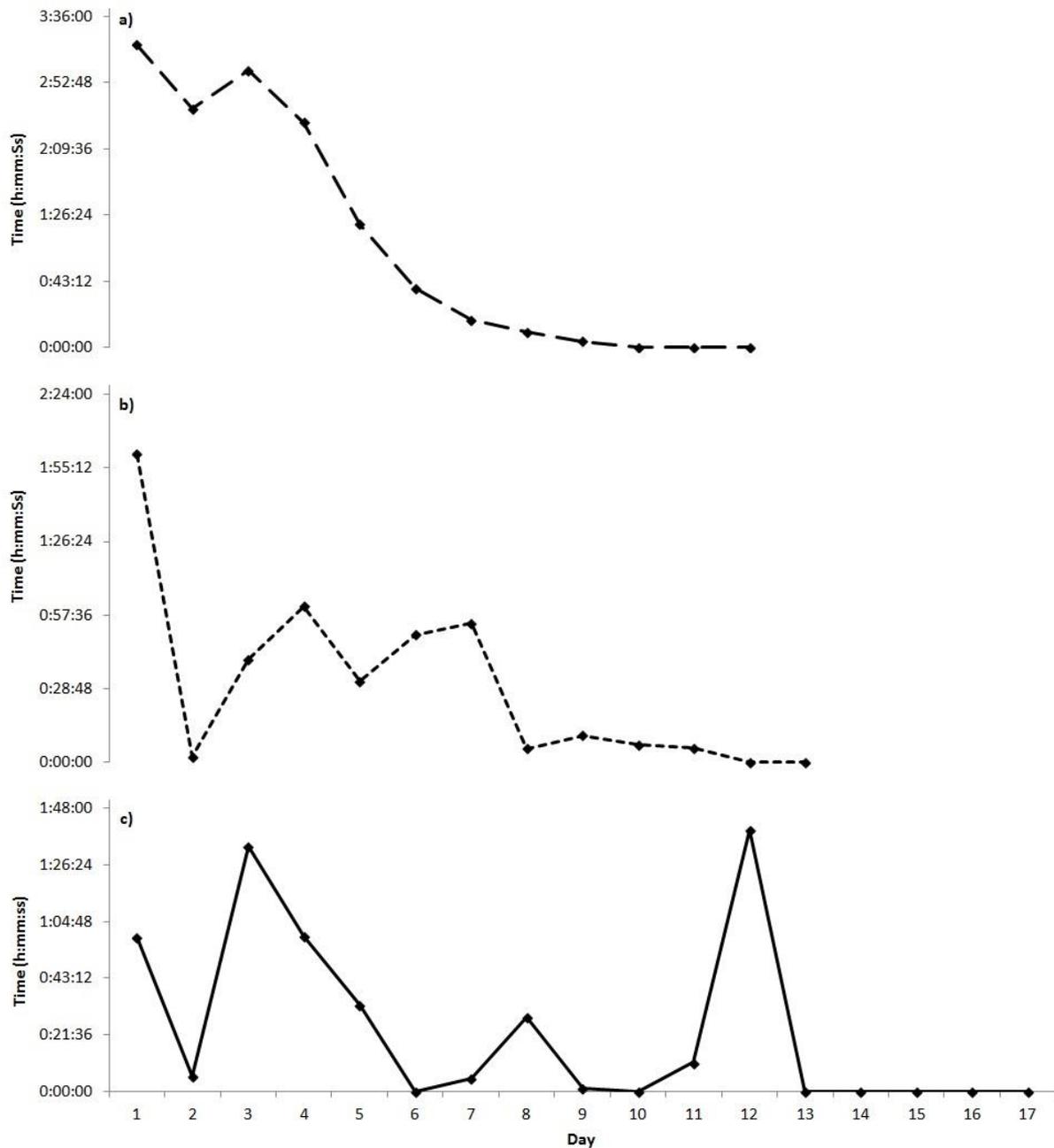


Figure 11. Time at feed troughs per day for three sheep that died and were positive for salmonella.

4.2 Intervention

4.2.1 August 2013: Shed versus paddock with hay and/or pellets

Sheep were monitored in the shed for 6 days; however, there was a power failure on day 6 resulting in an incomplete set of data for this day. Additionally, the feedlot manager determined that this group of sheep were not performing well in the shed and removed these sheep into the paddocks from day 6.

The average time per day spent at the feed troughs was not different between treatments ($p>0.05$) (Table 7; Fig. 12). Sheep alive at the end of the feedlot period spent an average of 52m 27s longer at the feed troughs per day than sheep that died during the feedlotting period ($p<0.05$). There was no difference in the time spent per visit to the feed troughs between sheep alive at the end of the feedlot period and sheep that died during feedlotting ($p>0.05$). There are many uncontrolled variables between these sheep and those monitored previously, such that a valid statistical comparison cannot be undertaken. However, these sheep appeared to have fewer, and shorter, visits per day to the feed troughs, compared to the previous groups, and therefore a shorter total time per day at the feed troughs.

Table 7. Comparison of time at feed troughs and number of visits between treatment groups in all sheep and sheep dead or alive at the end of the feedlot period. Shed pellets* corrected for the extra day in the shed at the beginning of the trial when the other two groups were housed in paddocks.

	Mean total time	Mean number of visits per day	Mean time per day	Mean time per visit
ALL SHEEP				
Shed Pellets*	4:56:22 ± 2:51:47	29.7 ± 15.3	1:14:06 ± 0:42:57	0:02:26 ± 0:00:36
Paddock Pellets	4:49:41 ± 2:23:19	29.9 ± 13.8	1:12:25 ± 0:35:50	0:02:26 ± 0:00:39
Paddock Hay Pellets	4:30:32 ± 2:31:29	27.3 ± 13.5	1:07:38 ± 0:37:52	0:02:28 ± 0:01:01
ALIVE				
Shed Pellets*	5:02:53 ± 2:50:19	30.4 ± 15.1	1:15:43 ± 0:42:35	0:02:27 ± 0:00:36
Paddock Pellets	4:54:23 ± 2:20:24	30.4 ± 13.4	1:13:36 ± 0:35:06	0:02:26 ± 0:00:37
Paddock Hay Pellets	4:48:53 ± 2:27:51	29.1 ± 13.3	1:12:13 ± 0:36:58	0:02:30 ± 0:01:03
DEAD				
Shed Pellets* (n=5)	1:33:03 ± 0:54:22	10.55 ± 5.24	0:23:16 ± 0:13:36	0:02:03 ± 0:00:26
Paddock Pellets (n=3)	0:41:50 ± 0:42:15	3.6 ± 3.2	0:10:27 ± 0:10:34	0:03:10 ± 0:02:10
Paddock Hay Pellets (n=21)	2:27:19 ± 1:55:43	15.7 ± 9.1	0:36:50 ± 0:28:56	0:02:12 ± 0:00:43

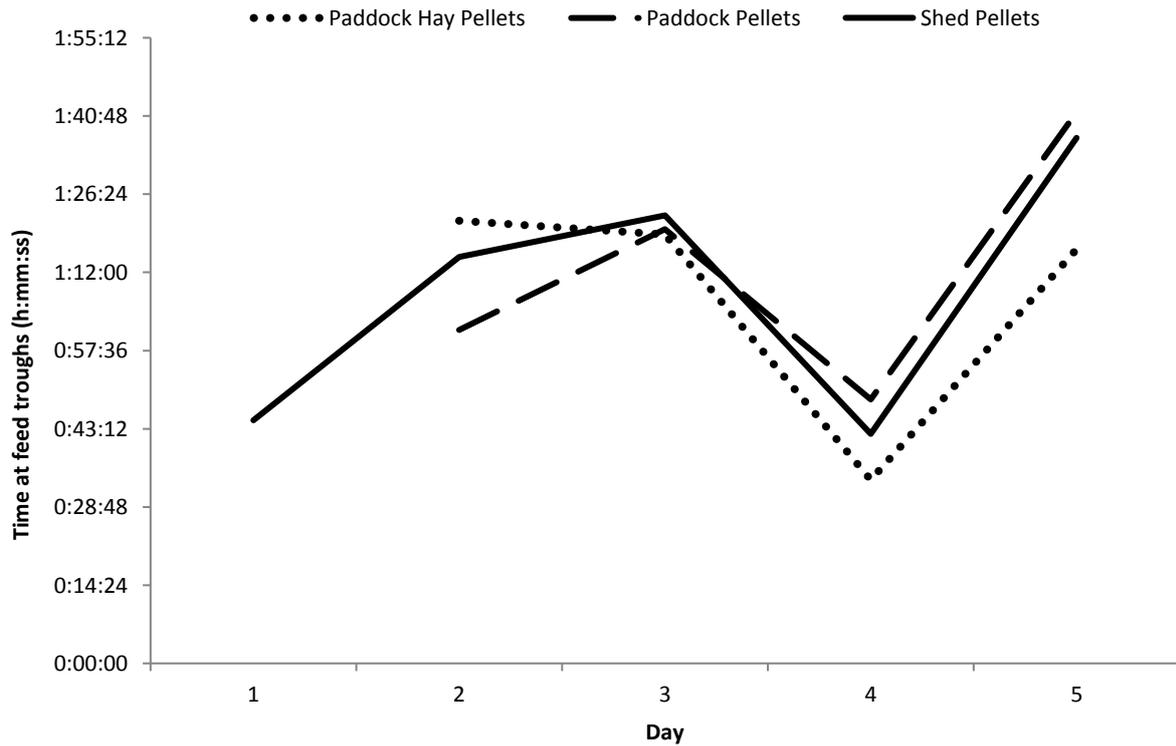


Figure 12. Mean time per day at the feed troughs for each treatment.

Of the sheep monitored, 21 (13 %) died from the *Paddock Hay Pellets* group, from day 6 onwards, 3 (1.9 %) died from the *Paddock Pellets* group, and 5 (3.1 %) died from the *Shed Pellets* group (Fig. 13). Of the sheep that died, all except five (which were either too decomposed for post mortem or had been removed by feedlot staff) were diagnosed with Salmonella/inanition (Fig. 14).

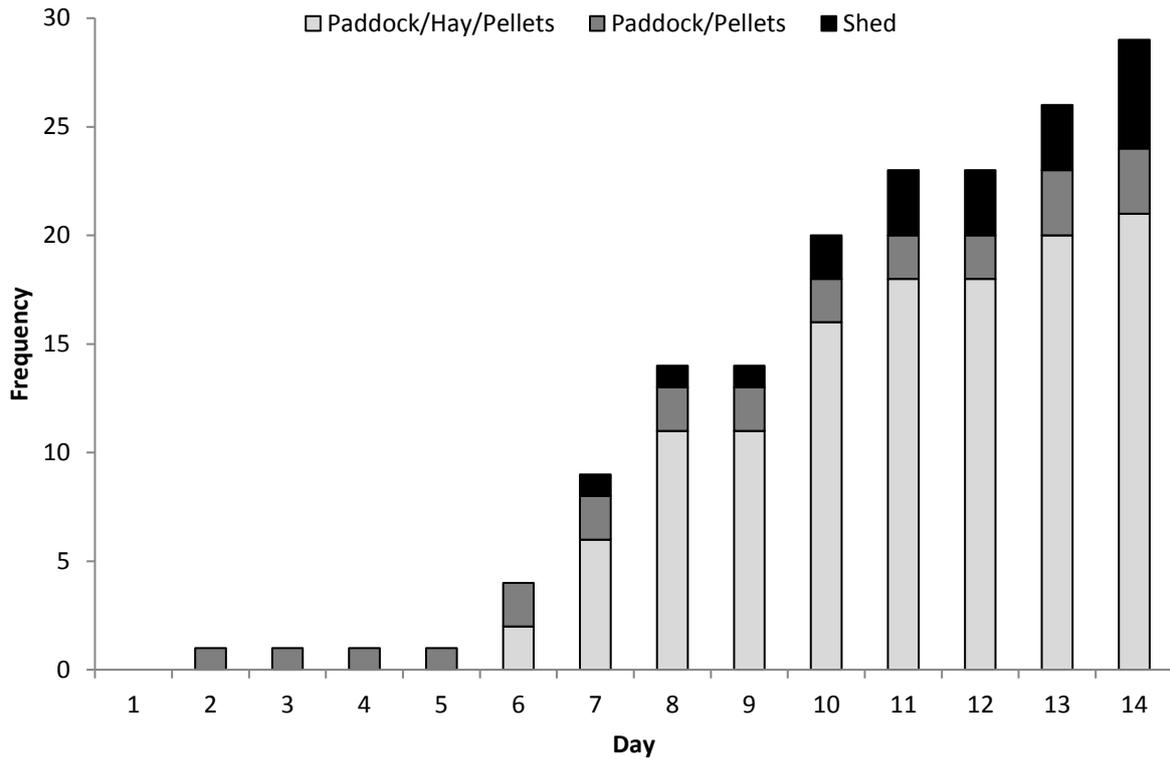


Figure 13. Cumulative frequency of mortality during August 2013

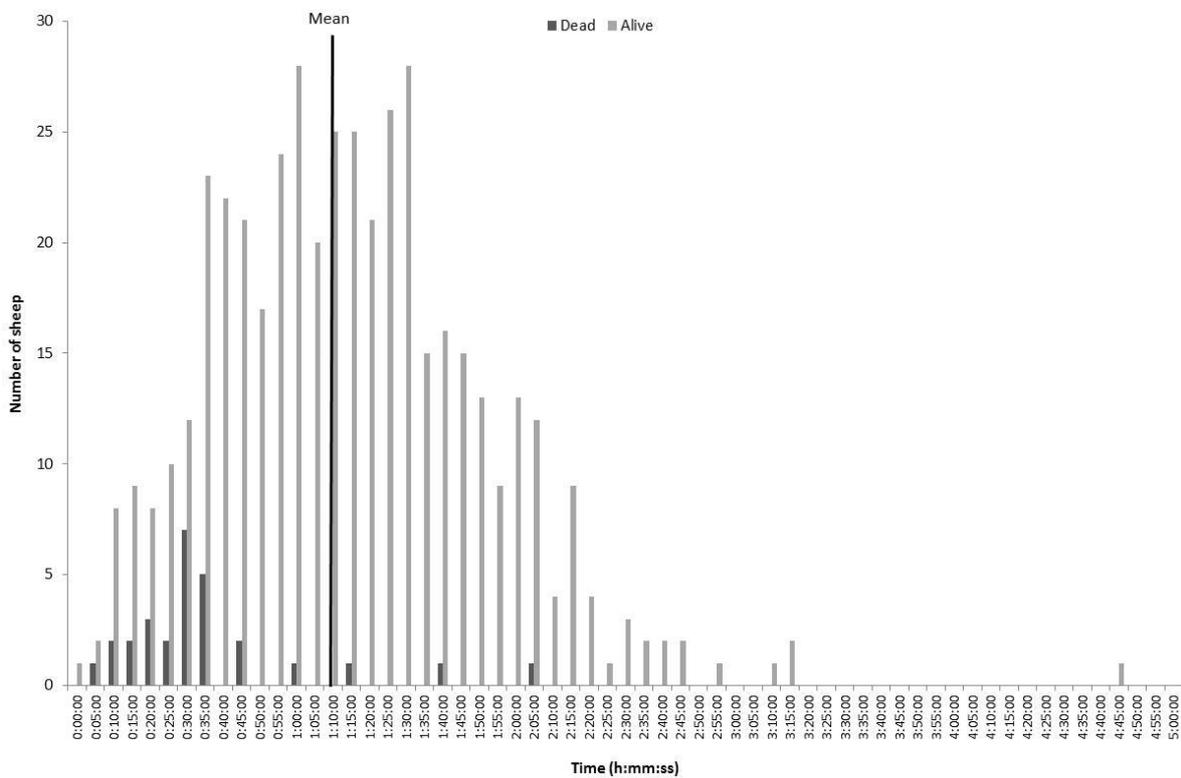


Figure 14. Histogram of the mean average time spent at the feed troughs in August 2013. Sheep that died are represented by dark bars. The daily mean is shown.

The previously defined minimum daily time for feed trough attendance of 28m 5s was used to consider which animals were inappetent. The percentages of sheep from this experiment

defined as inappetent on day 1 and 2 were higher than for the previously monitored groups. There was also an increase in the percentage of inappetent sheep on day 4 in *Paddock Hay Pellets* group compared to the *Paddock Pellets* and *Shed Pellets* groups ($p < 0.05$). There was no other difference between treatments on other days (Fig 15.).

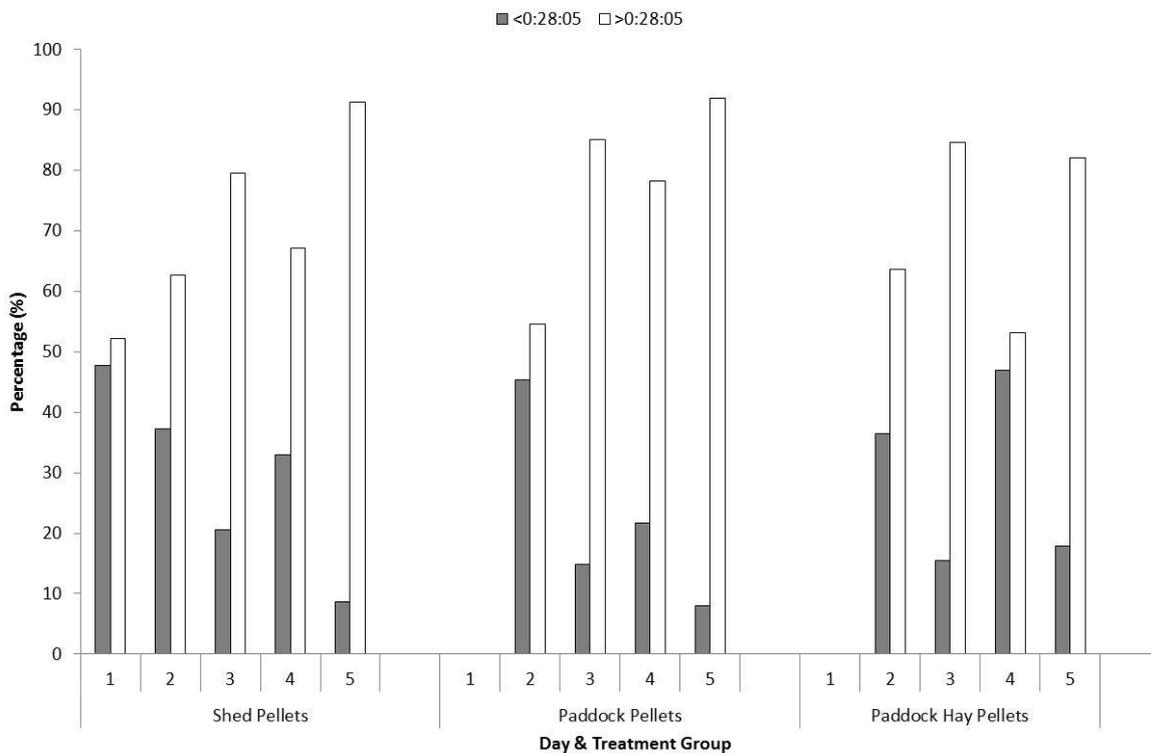


Figure 15. Percentage of sheep in the three treatment groups attending the feed troughs for less than or greater than 28m 5s

4.2.2 December 2013: Shed versus paddock with hay and/or pellets

Sheep in the *Shed Pellet* group visited the feed troughs more often and spent longer at the feed troughs per day than sheep in the *Paddock Pellets* and *Paddock Hay Pellets* groups ($p < 0.05$) (Table 8). However, sheep in the *Paddock Hay Pellets* group spent the most time per visit at the feed troughs ($p < 0.05$). There was no difference between sheep in the *Shed Pellets* and *Paddock Pellets* group in the mean time per visit. All three groups of sheep in this experiment spent longer per visit, and per day, than the August 2013 cohort (4.2.1) (Fig. 16).

Table 8. Comparison of time at feed troughs and number of visits between treatment groups. Shed pellets* corrected for the extra two days in the shed at the beginning

	Mean total time	Mean number of visits per day	Mean time per day	Mean time per visit
Shed Pellets*	43:58:24 ± 13:36:15	30.2 ± 8.9	1:59:56 ± 0.:37:04	0:04:02 ± 0:00:55

Paddock Pellets	38:07:02 ± 14:27:28	26.2 ± 9.2	1:43:58 ± 0:39:25	0:03:53 ± 0:01:03
Paddock Hay Pellets	40:32:00 ± 12:43:49	27.1 ± 8.7	1:50:40 ± 0:34:24	0:04:07 ± 0:00:53

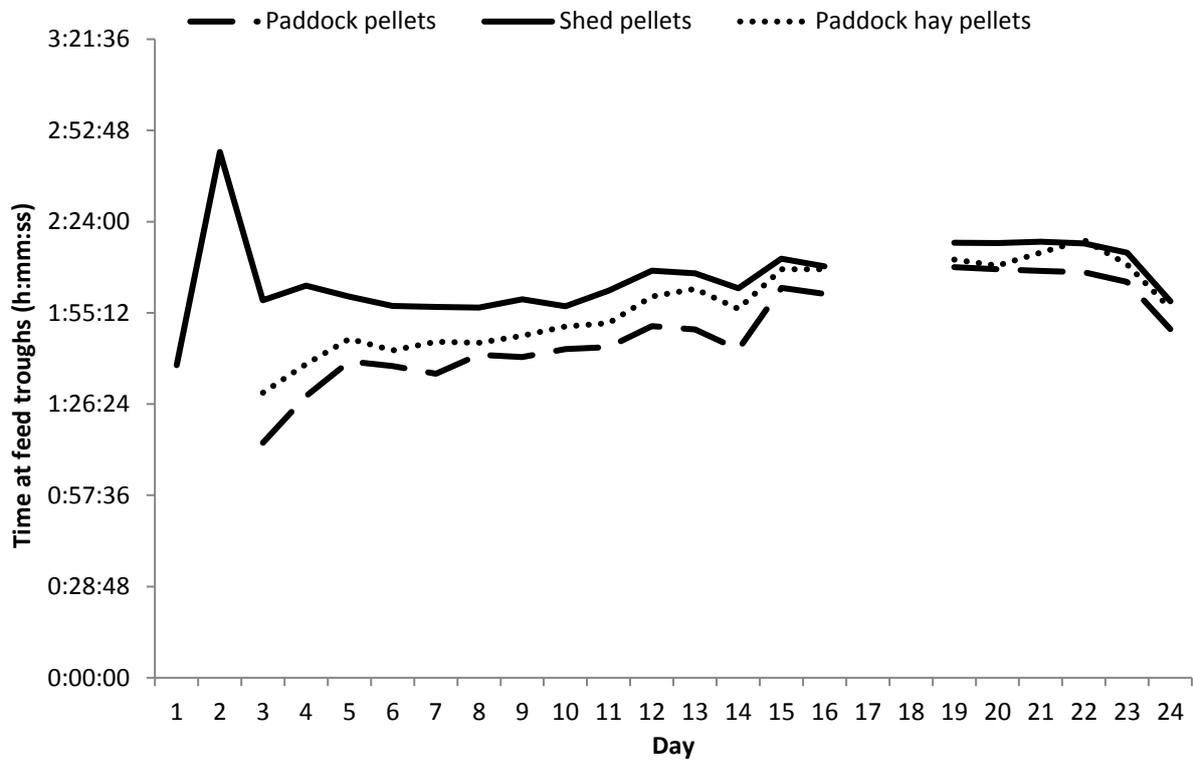


Figure 16. Mean daily time per day at the feed troughs. There was a power failure on days 17 and 18, so no data could be recorded. Only the Shed pellets group were recordable for days 1 and 2.

Mortality was 0.98 % (6/612) diagnosed as due to Salmonella/inanition, with three deaths in the *Paddock Hay Pellets* groups, one death in the *Shed Pellets* group, and two deaths in the *Paddock Pellets* group. For the total time the sheep were present in the experiment, the average daily time at the feed troughs was less than the defined minimum (Fig. 17), although the three Paddock Hay Pellets sheep had spent longer than an hour at the feed troughs on the first day they entered the shed.

At discharge from the feedlot to the ship, 15 sheep were considered by feedlot staff as not fit for transport due to poor body condition or injury/disease/lameness (eight *Paddock Pellets*, four *Shed Pellets* and three *Paddock Hay Pellets*). Six of these sheep fit the definition of inappetent for the monitored period in the shed.

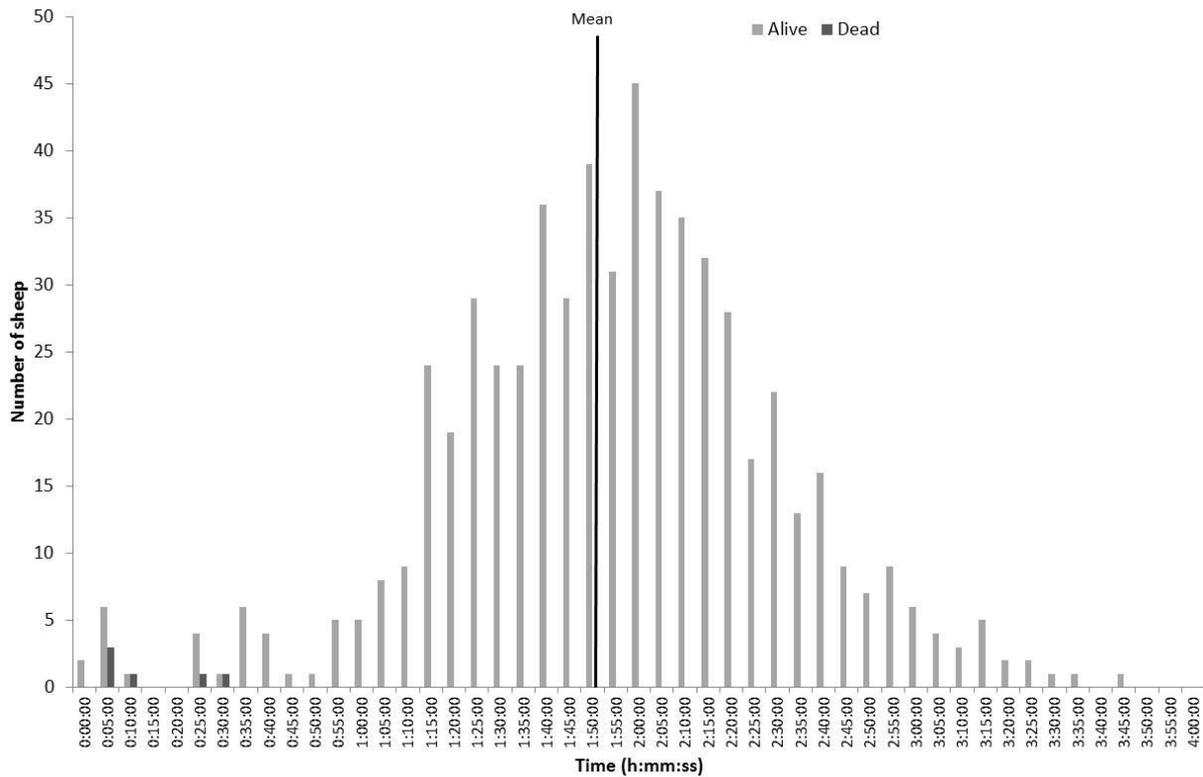


Figure 17. Histogram of the mean daily time spent at the feed troughs in December 2013. Sheep that died are represented by dark bars. The daily mean is shown.

On day one in the shed, 32.3% of the sheep in the *Shed Pellets* group were defined as inappetent, attending the feed troughs for less than 28m 5s. By day three this had decreased to 10.8%. When the *Paddock Pellet* group were moved to the shed (on day 3), 28.1 % were considered inappetent; however, when the *Paddock Hay Pellet* group were moved to the shed (day 3), 13.0 % were considered inappetent (Fig. 18). The pattern of feed trough attendance for the Paddock Pellets group was thus similar to the Shed Pellets group, jut delayed by the two days in the paddock.

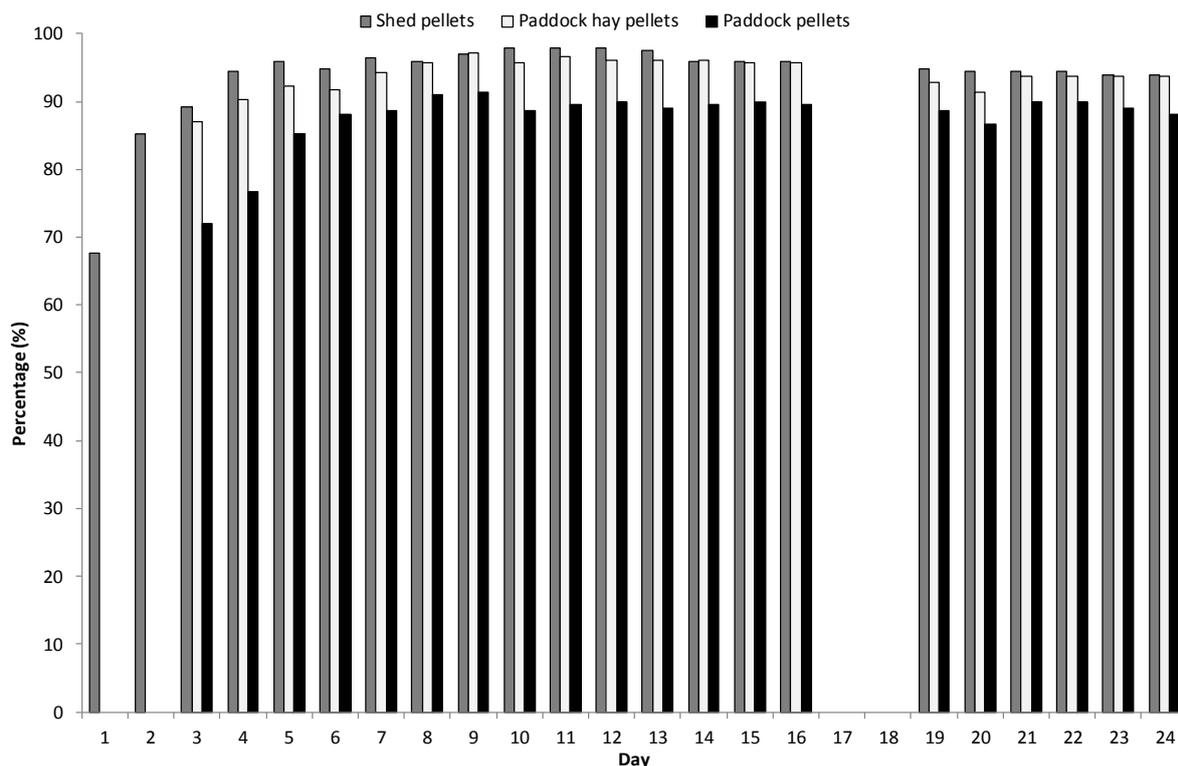


Figure 18. Percentage of sheep in the three treatment groups attending the feed troughs for greater than 28m 5s. Power failure on days 17 and 18. Only the shed pellets group were recordable for days 1 and 2.

4.2.3 January 2014: Pellets and Oats

There was no difference in total time at feed troughs, total number of visits to the feed troughs, average time per day spent at the feed troughs, or mean time per visit between the *Pellets and Oats* groups ($p > 0.05$) (Table 9; Fig. 20). Sheep spent 1h 48m 17s \pm 47m 33s per day at the feed troughs (mean \pm SD). Although there was no treatment difference in time spent at feed troughs, sheep in pen 3 spent less time at the feed troughs than sheep in pens 1 and 4 ($p < 0.01$). There was no interaction between treatment and day observed. There was no mortality from this cohort of sheep (Fig. 19).

Table 9. Comparison of time at feed troughs and number of visits between treatment groups.

	Mean total time	Mean number of visits per day	Mean time per day	Mean time per visit
Pellets	7:20:35 \pm 3:00:07	36.5 \pm 15.3	1:50:09 \pm 0:45:02	0:03:04 \pm 0:00:47
Oats	7:05:57 \pm 3:19:16	35.0 \pm 15.5	1:46:29 \pm 0:49:49	0:03:04 \pm 0:00:51

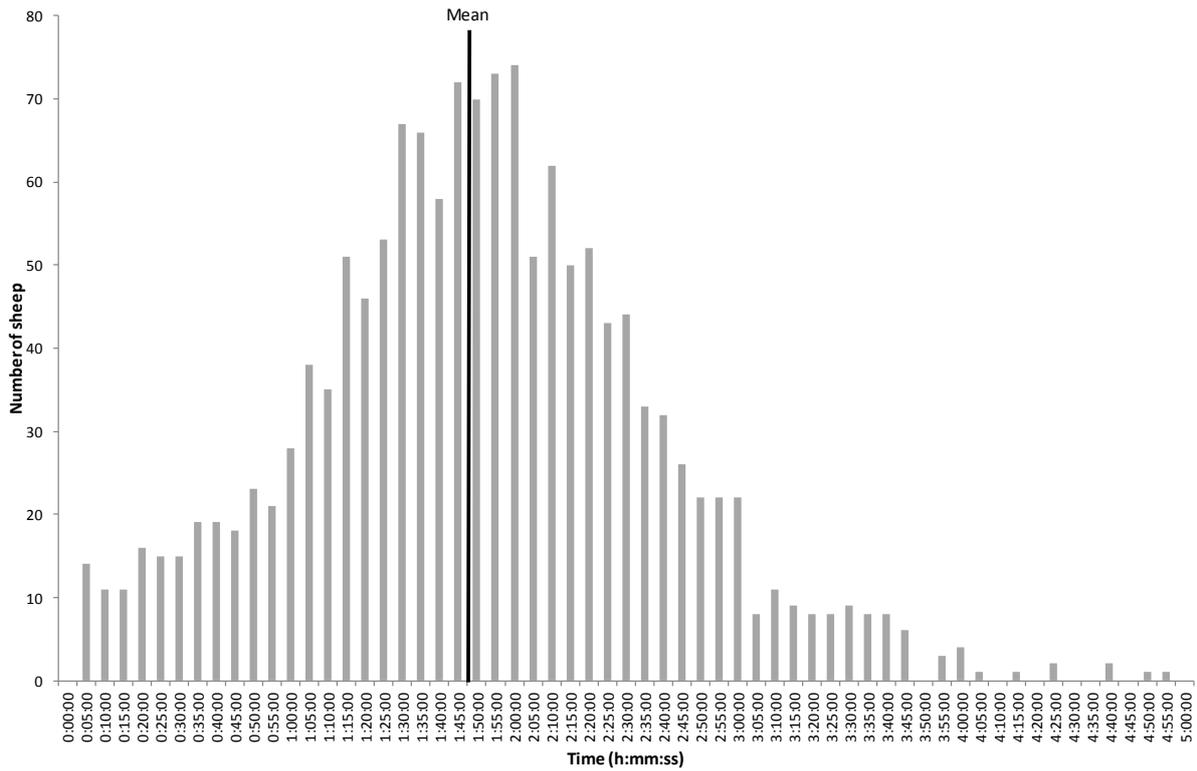


Figure 19. Histogram of the daily mean time spent at the feed troughs in January 2014. The mean daily average is shown.

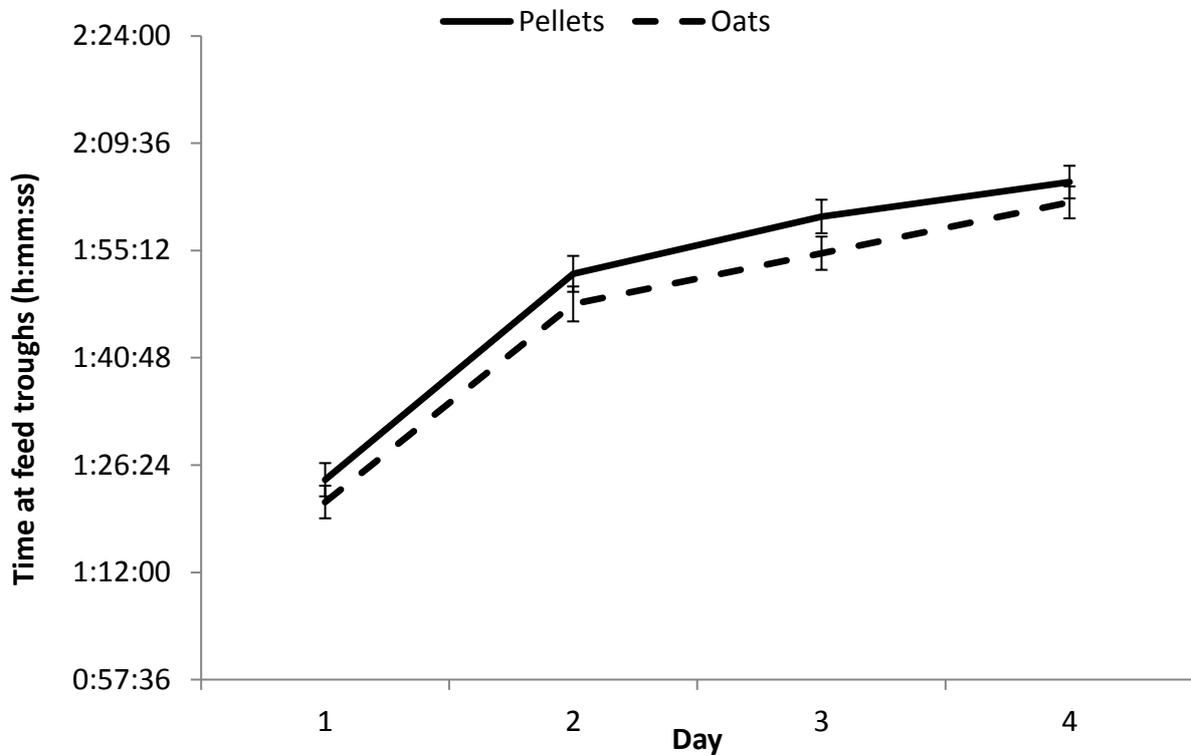


Figure 20. Mean time at feed troughs per day for each treatment.

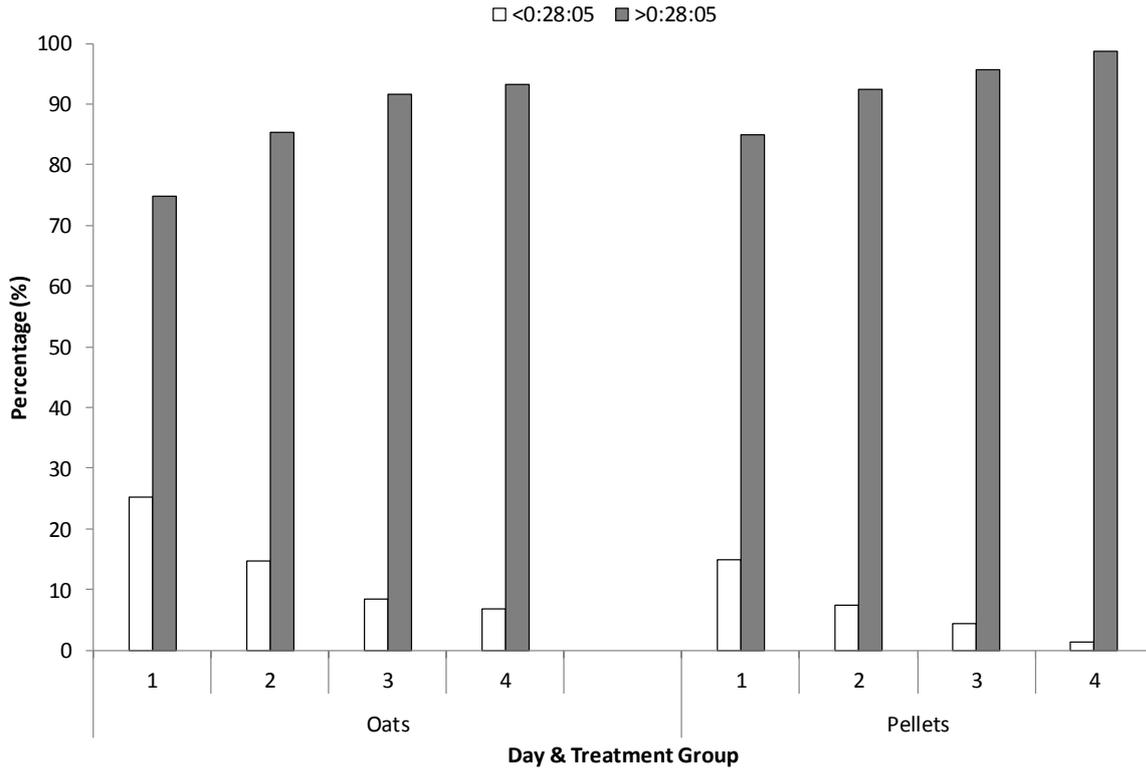


Figure 21. Percentage of sheep in the two treatment groups attending the feed troughs for less than or greater than 28m 5s.

4.2.4 August 2014: Pellets and Chaff

The sheep spent 1h 06m 36s ± 30m 22s per day at the feed troughs (mean ± SD). Sheep in the *Chaff* group visited the feed troughs more often and spent more time per day at the feed troughs than sheep in the *Pellets* group ($p < 0.05$). However, sheep in the *Pellets* group spent more time per visit at the feed troughs compared to the *Chaff* group ($p < 0.05$) (Table 10; Fig. 23). There was a fault with three out of six feed troughs in one pen of the *Pellets* group and this data has been removed from the analysis. There was no mortality from this cohort of sheep (Fig. 22).

Table 10. Comparison of time at feed troughs and number of visits between treatment groups.

	Mean total time	Mean number of visits per day	Mean time per day	Mean time per visit
Pellets	6:24:44 ± 2:53:50	23.13 ± 3.56	1:04:07 ± 0:28:58	0:02:49 ± 0:00:47
Chaff	6:47:19 ± 3:06:58	26.00 ± 3.93	1:07:53 ± 0:31:10	0:02:39 ± 0:00:45

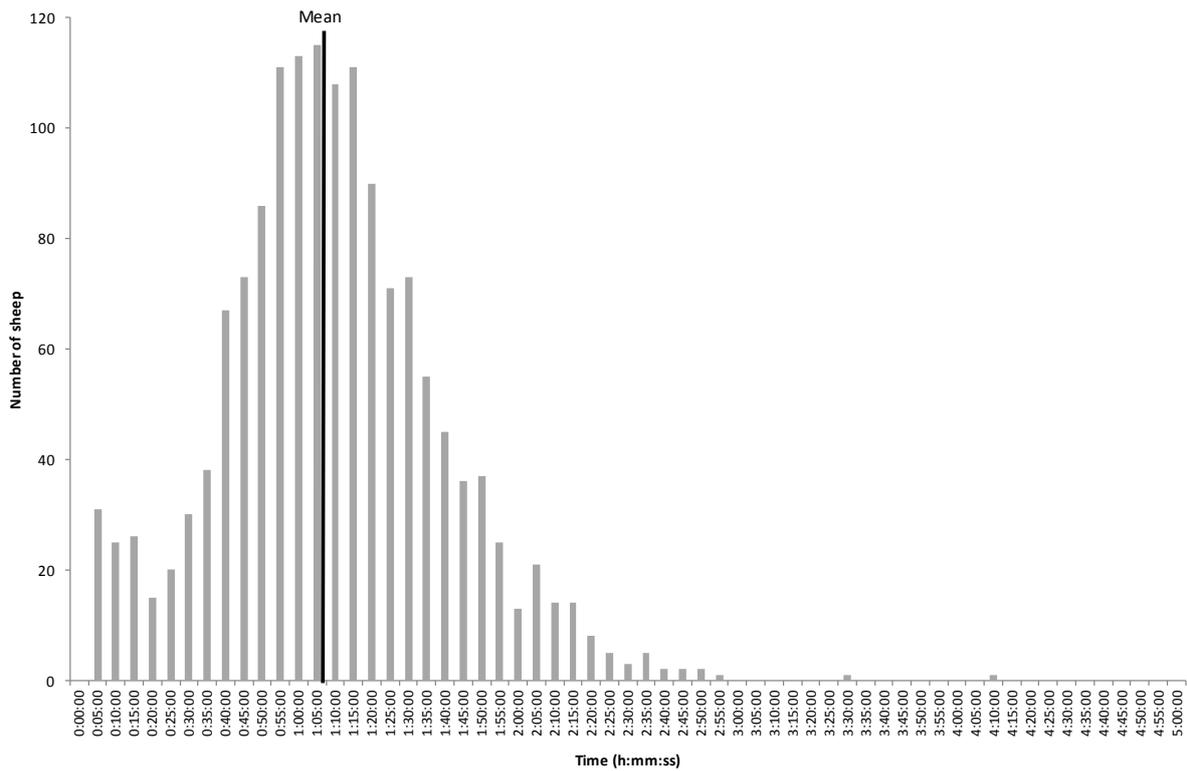


Figure 22. Histogram of the daily average time spent at the feed troughs in August 2014. The mean daily average is shown.

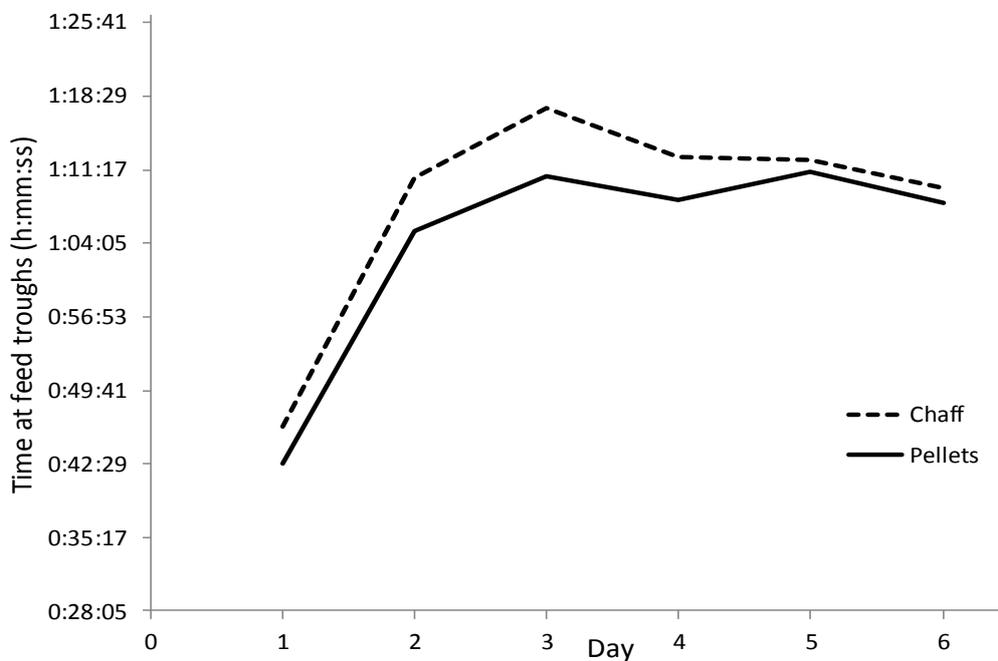


Figure 23. Average time at feed troughs per day for *Chaff* and *Pellet* treatment groups. *The data from one group of sheep in the pellets group has been removed due to faulty antennae in three feed troughs in pen 2.

There was no difference between the treatments in the percentage of sheep defined as inappetent ($p>0.05$) (Fig. 25).

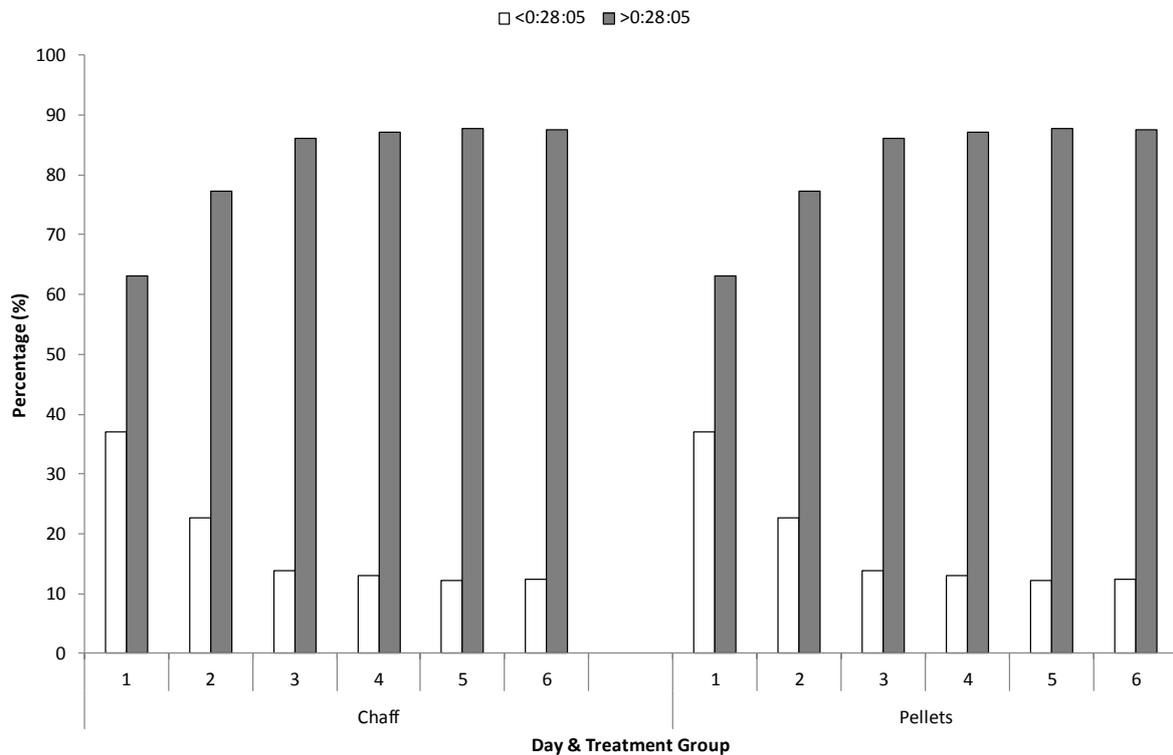


Figure 24. Percentage of sheep in the two treatment groups attending the feed troughs for less than or greater than 28m 5s

4.2.5 October 2014: Pellets and Chaff

The average time spent at the feed troughs per day for all sheep was 2h 00m 35s \pm 40m 12s. Sheep in the *Chaff* group visited the troughs more frequently than sheep in the *Pellets* group; however, sheep in the *Pellets* group spent more time per visit at the feed troughs ($p<0.05$) (Table 11, Figure 25). There was no difference between total time and average time per day spent at the feed troughs between treatments. There was a fault with the antennae in one feed trough (1 out of 6) in Pen 2 (*Chaff* group). Data for this pen was removed from the analysis. There was no mortality from this cohort of sheep (Fig. 26).

Table 11. Comparison of time at feed troughs and number of visits between treatment groups.

	Mean total time	Mean number of visits per day	Mean time per day	Mean time per visit
Pellets	17:59:40 \pm 6:08:14	37.4 \pm 12.5	1:59:58 \pm 0:40:55	0:03:17 \pm 0:00:47
Chaff	18:23:49 \pm 5:23:06	44.5 \pm 13.9	2:02:39 \pm 0:35:54	0:02:51 \pm 0:00:42

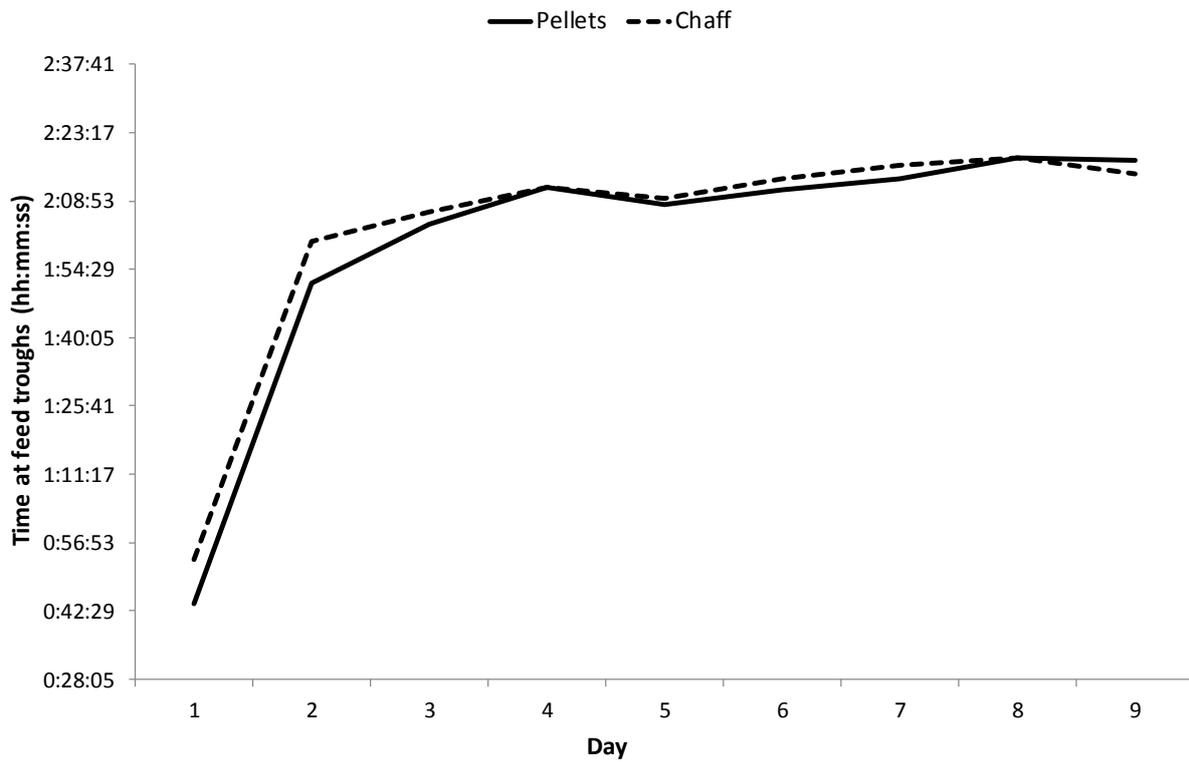


Figure 25. Average time at feed troughs per day for each treatment.

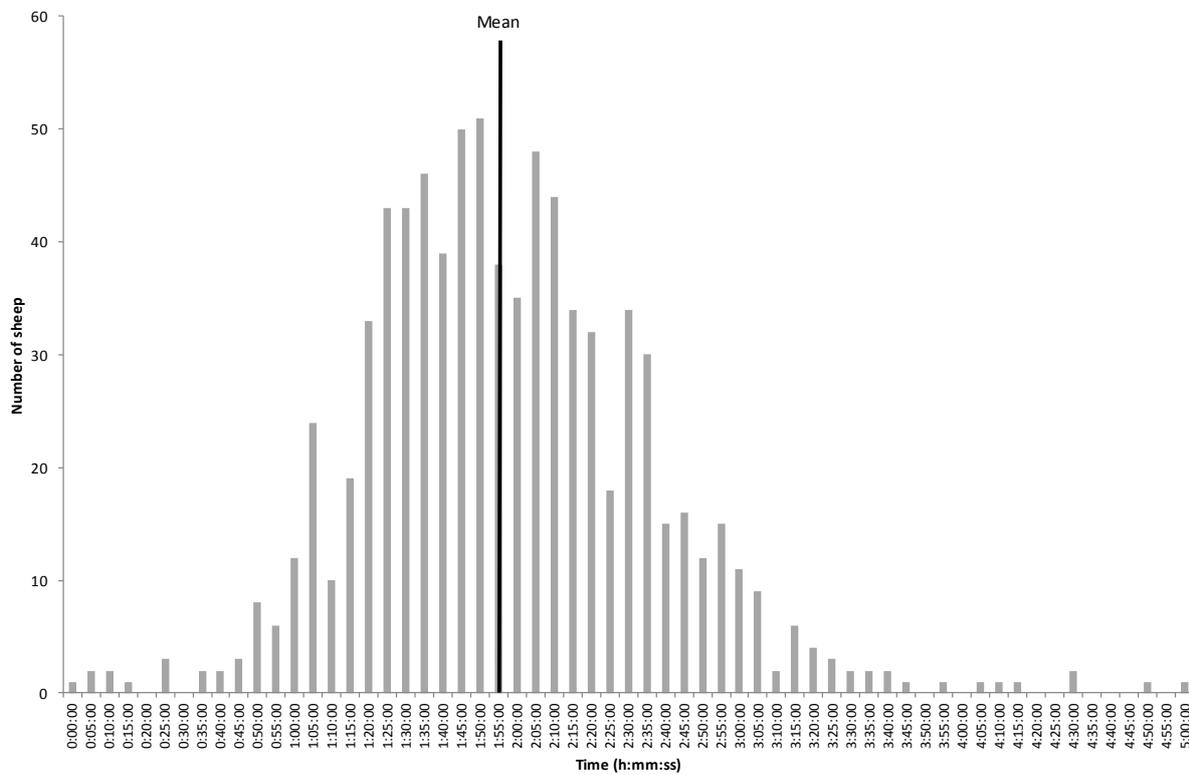


Figure 26. Histogram of the daily average time spent at the feed troughs in October 2014. The mean daily average is shown.

There were less sheep defined as inappetent on day 1 in the *Chaff* group compared to the pellets group ($p < 0.05$) (Fig. 28).

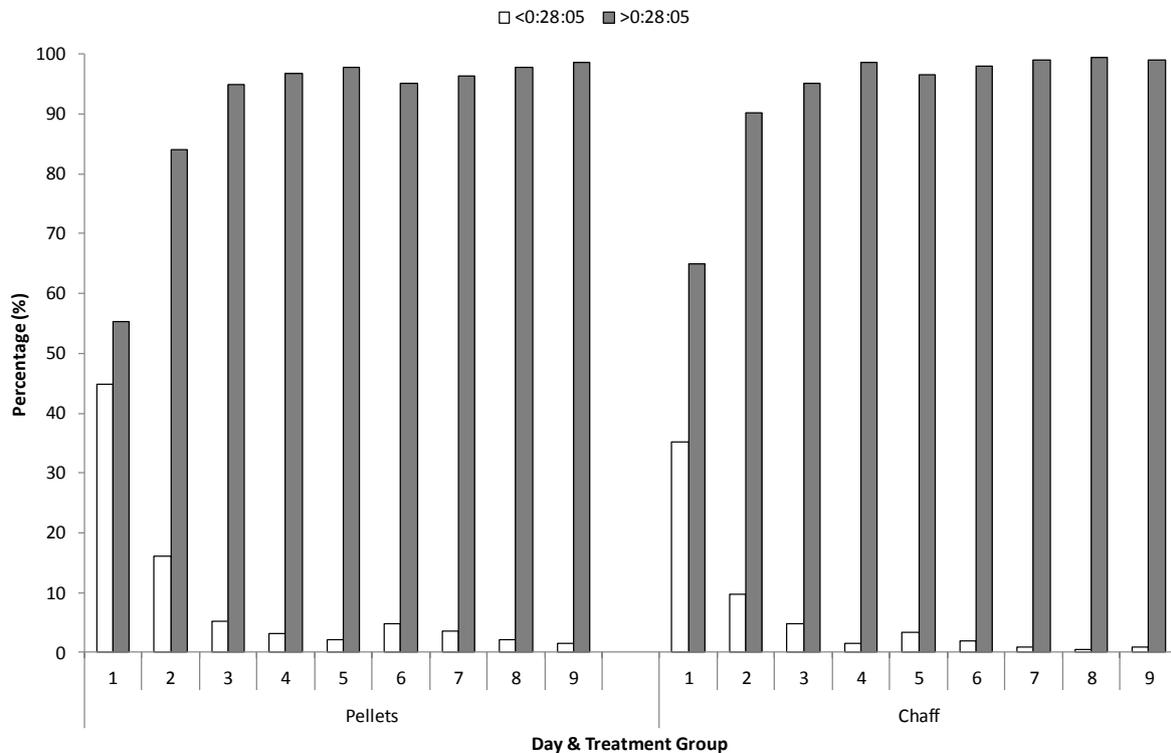


Figure 27. Percentage of sheep in the two treatment groups attending the feed troughs for less than or greater than 28m 5s. Data from pen 2 has been excluded.

4.3 Preparation

4.3.1 February 2015: On farm pellets or hay

The average time spent at the feed troughs per day for all sheep was 2h 24m 35s \pm 40m 12s. Twelve sheep did not arrive at the feedlot and their fate is unknown. The sheep in all groups spent an average daily time at the feed troughs that was nearly an hour longer than the average for the previously monitored sheep (Table 7, Fig. 28). Sheep in the *Control* group spent more time at the feed troughs on days 2 and 3 compared to *Hay* and *Pellet* groups ($p < 0.05$). The *Pellets* group spent less time at the troughs on day 1 compared to *Hay* and *Control* groups ($p < 0.05$).

Sheep in the *Pellets* group spent less total and average time per day at the feed troughs and visited the feed troughs less times that sheep in the *Hay* or *Control* groups ($p < 0.01$). However, there is no statistical difference in average time per visit to the trough between the three treatments ($p > 0.05$). There were no mortalities recorded for the *Hay*, *Pellet* or *Control* group.

Only two sheep were defined as inappetent for the whole feedlot period with their average daily time at the feed troughs less than 28m 5 s; one was from the *Control* group and the other from the *Hay* group (Fig. 29).

Table 7. Comparison of time at feed troughs and number of visits between treatment groups.

	Mean total time	Mean number of visits per day	Mean time per day	Mean time per visit
Control	15:15:32 ± 4:19:25	51.7 ± 15.1	2:32:35 ± 0:43:14	0:03:01 ± 0:00:38
Hay	14:39:48 ± 4:01:26	50.2 ± 13.3	2:26:38 ± 0:40:14	0:02:58 ± 0:00:37
Pellets	13:27:15 ± 3:28:22	46.9 ± 13.5	2:14:33 ± 0:34:44	0:02:57 ± 0:00:35

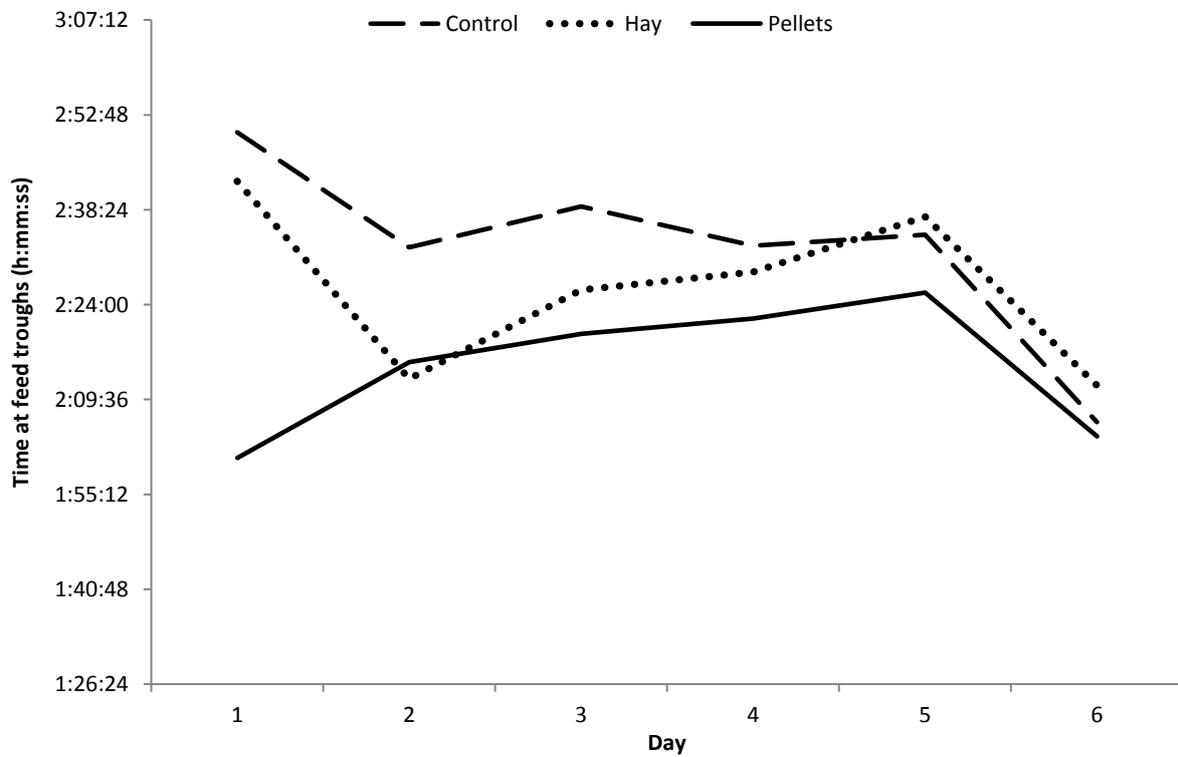


Figure 28. Mean time spent at the feed troughs each day per treatment.

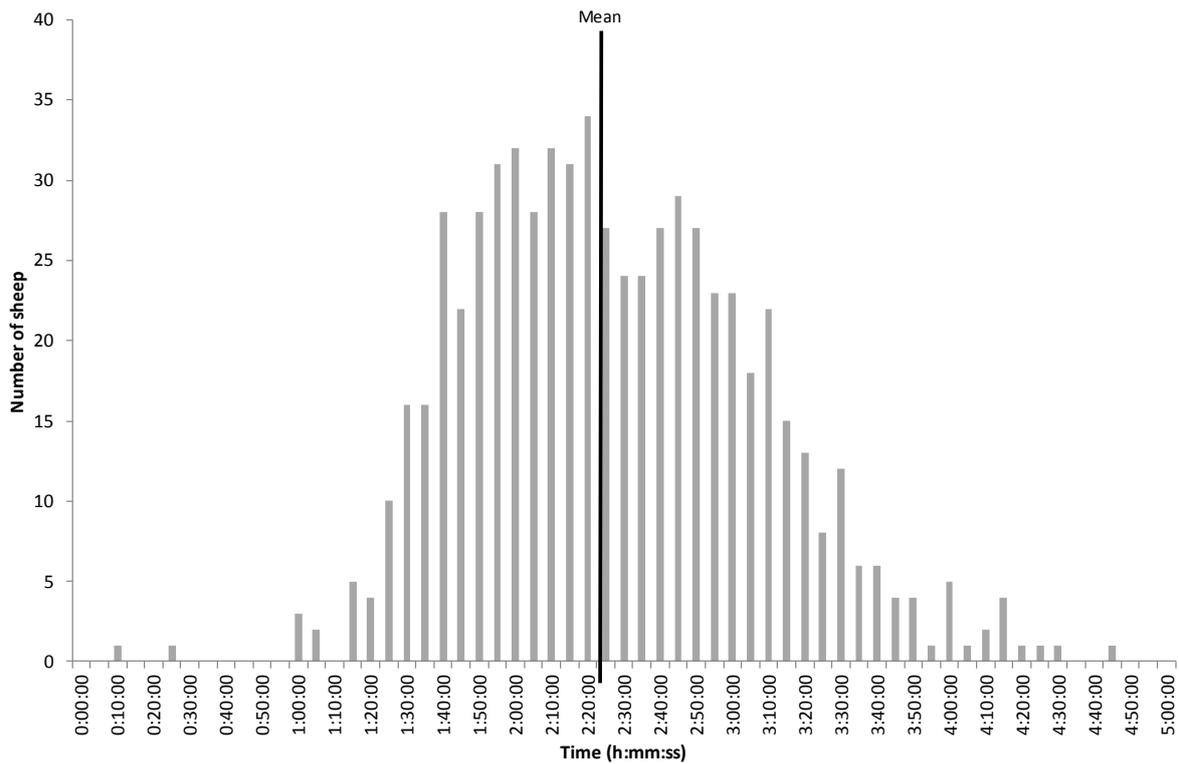


Figure 29. Histogram of the daily average time spent at the feed troughs in February 2014. The mean daily average is shown.

There is a significant decline in the percentage of sheep defined as inappetent in the *Hay* group on day 2, compared to other days and compared to other treatments. There is no significant difference between other treatments, or days within treatments (Fig. 30).

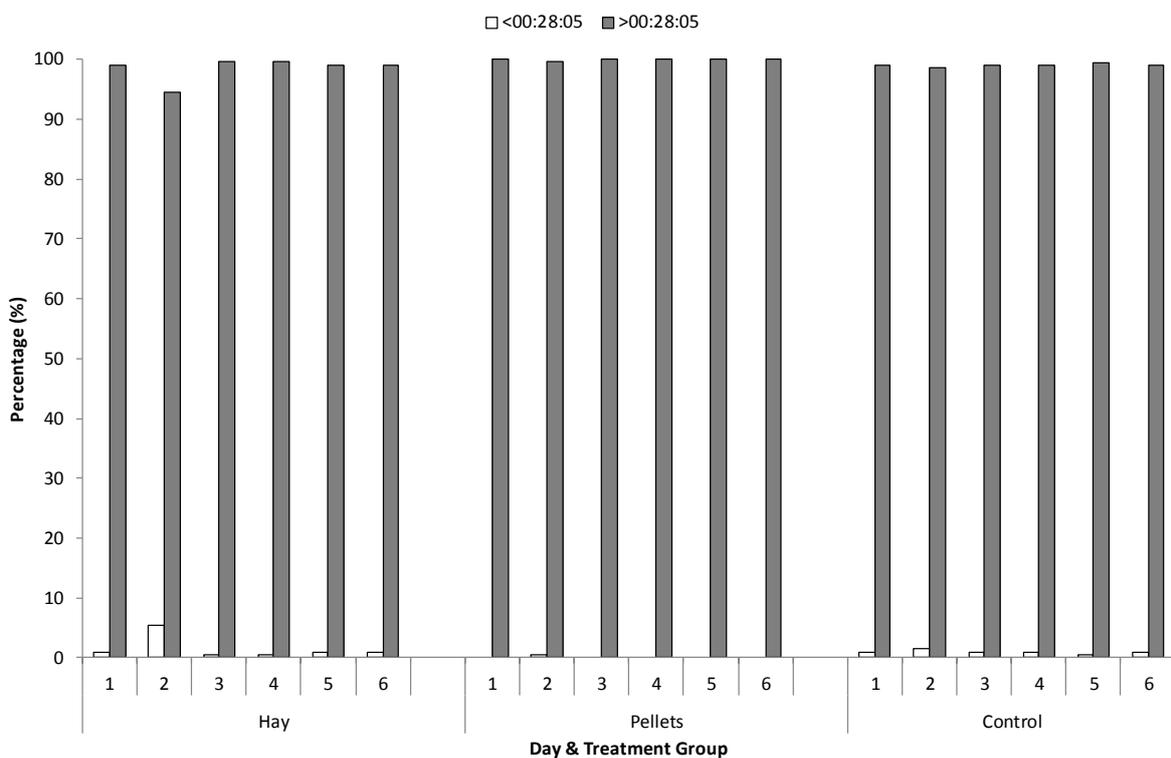


Figure 30. Percentage of sheep in the two treatment groups attending the feed troughs for less than or greater than 28m 5s. Day 6 does not include a full 24 hours of data.

5 Discussion

Over 8000 sheep were monitored at the pre-embarkation feedlot in the first 10 months of the project, from a variety of origins, breeds and lines. While these animals are only a small percentage of those that were shipped, they were considered representative of the usual range of sheep shipped live from Western Australia. The RFID tracking antennae were successful in detecting individual sheep at the feed and water troughs, as confirmed by the data recovered, and the detection of tags using the RFID wand at exit from the feedlot. The system was able to quantify the amount of time individual sheep spent at the feed and water troughs within a determined time frame. The system generated data that could be used to assess feeding behaviour in the sheep. Sheep fed on pellets have been observed taking a mouthful of pellets then moving their head away from the trough while they chew, then returning for another mouthful, while some animals keep their head in the trough throughout. Those animals that moved their head away between mouthfuls were still detected using our system as the parameters could be set such that a feeding instance could be as short as 5 s, the time it might take a sheep to put its head into the trough and take a mouthful of pellets. This resulted in higher total time and numbers of feeding occasions than setting a longer minimum time.

There were significant differences in the time spent at the feed troughs between sheep that were alive or dead at the end of the feedlotting period, in average time per visit, average number of visits and average time per day. However, there was no difference in the amount of time these sheep spent at the water troughs. This system, for recording time at feed and water troughs, has merit in identifying individual animals which are not attending the feed and water troughs. Whilst this monitoring system is expensive and time consuming to implement, it can reveal much about feeding behaviour. The system could be used with automatic drafting systems or other non-intrusive management that remove such animals for different feed or attention. However, the benefits of any such change in management would need to be weighed against the costs of disruption, and the technology is not suitable for general installation.

5.2 Incidence of inappetence in specific lines of sheep

The tracking of multiple cohorts of sheep, from different sources, at different times of year, means that it is difficult to directly compare the groups. However, the mean daily average time spent at the feed troughs, for the 8206 sheep in the initial monitoring experiments was over an hour and a half (1:35:41 ± 0:33:48 h:m:s). It was not possible, due to the low mortality rate, to effectively calculate the minimum daily time at the feed trough that was directly related to a higher risk of mortality (Appendix 1). Therefore, based on observations of the sheep including change in BCS, the pattern of feeding, and mortality, inappetence was defined as sheep spending less time per day at the feed troughs than 2 standard deviations below the mean for the whole group, 28m 5s. As a general guide, it appears that a sheep fed in a shed on pellets that attends the feed trough for less than an hour per day is below

average and may not be feeding adequately, while a sheep that attends the feed trough for less than half an hour can be considered inappetent. Rice et al. (2016) have developed a similar conclusion regarding feeding time of Merino lambs; that those animals spending less than 30 minutes at the feed trough per day were categorised as “shy-feeders”.

Based on the definition of less than 2SD below the mean, it took until day six in the feedlot for less than 5% of animals to be inappetent.

Different groups of sheep were monitored for varying lengths of time, for as short as six days, up to one group housed for 31 days. The sheep came from multiple origins and lines, and it was not possible to retrospectively assess any farm of origin effects. However, at no time, even in the group housed for a month, were there no sheep defined as inappetent. It was not necessarily the same sheep each day which were not feeding, and inappetence on any day was not necessarily predictive of death. The data was complicated by the exit of different groups on different days, but it appears that after five days in the feedlot an acceptably high percentage of sheep were attending the feed troughs for more than the defined minimum. Feedlotting the sheep for longer than five days may not provide further benefit in terms of significantly more sheep eating, although the group which remained in the feedlot for a month were close to 100% eating well by the time of exit.

Although retrospectively we have defined inappetence based on the amount of time spent at the feed troughs, and the majority of animals which did die were those not attending the feed troughs for average daily periods, it became apparent through the monitoring of the large groups of sheep that removing sheep that had not attended the feed trough on any given day would mean disrupting the large group. The linear discriminant analysis (Appendix 1) showed that feeding time on day 2 is important in predicting outcome after 2-5 days have elapsed, and day 5 is important after 5 days have elapsed, but it remains very difficult to predict mortality outcome based on feeding time per day. Therefore, removing animals which might have been inappetent and possibly at risk of dying would result in misclassification of a huge part of the flock, neither practical nor economic.

Many of the sheep that did not eat well at the beginning of the feedlotting period commenced eating after several days, and while this project did not follow subsequent shipping and destination feedlot performance of the sheep, the overall feedback from the on ship veterinarians was of low mortality of these groups, indicating the majority would have been eating during subsequent stages of the export process. Norris et al. (1989b) reported that 80% of feedlot non feeders did commence eating within 5 days after being moved from a feedlot to a mock shipping system, and suggested that the added stress or movement or stimulation associated with a new system may have improved eating by the sheep.

Previous work investigating inanition has not considered water intake of the sheep, but in this current project, 7 out of 15 sheep that were not eating still were recorded at the water troughs. Post mortem examination of sheep diagnosed as dying from inanition has found fluid in the rumen (Richards *et al.*, 1989), which was presumed to indicate the animals were still drinking. This assumption may not be correct, in light of findings that dehydrated lambs still had the same weight of gastrointestinal contents as those with access to water, suggesting that the capacity for increased water absorption from the gastrointestinal tract in response to water deprivation is limited (Jacob *et al.*, 2006). However, dehydration has not been reported as a particular issue along with the inappetance; the long survival time of

animals not apparently accessing feed troughs indicated that the animals are still drinking, because dehydration will hasten mortality compared with deaths due to inanition alone.

5.3 The impact of time at feedlot on incidence of inanition and salmonellosis

The main cause of mortality was diagnosed as salmonellosis/inanition, and this finding corroborated previous work that indicates that the two syndromes are linked (Moore 2002). Intermittent feeding after exposure to salmonella may increase the likelihood of developing disease because the growth of salmonella increases when feed intake is decreased or interrupted for one or more days (Brownlie and Grau 1967), and feeding after a period of starvation is also associated with multiplication of *Salmonella* (Grau et al 1968, Frost and Samuel 1988). The incubation period for salmonellosis in cattle is reported as 1-4 days; House and Barnes (2009) state that that anorexia and fever are the first signs appearing 36-72 hours of *Salmonella* challenge, so that affected animals will then be recorded as not eating. In most challenge experiments the majority of mortalities are observed on days 3 – 10 post challenge and animals that survive 14 days following challenge are unlikely to die (House and Barnes 2009). Mortality attributed to salmonellosis in the experiments reported here started two to three days after the animal entered the shed, and was very obviously preceded in some cases by a decrease in time at the feed troughs (4.2.1; Fig.15). A shorter period in the shed might mean that affected animals do not die until they are on the ship, but those that survive after challenge at the feedlot appear to remain healthy on ship.

There may be a high potential for infection with *Salmonella* during transport and at the feedlot, when sheep may come into contact with faecal material and contamination. This was a greater problem for those sheep which spent a period in a paddock, in the WA winter, before entering the raised sheds, and subsequent assessment of the environment confirms the higher likelihood of contamination outside the sheds (data unreported here).

The other deaths included one group housed over summer where many of the animals were coughing, and pleuropneumonia with isolation of *Pasteurella* and *Mannheimia* was detected on post mortem examination. The contribution of pneumonia to feedlot mortality and morbidity is not commonly associated with pre-embarkation feedlots and shipping; however, it is described as a problem when sheep are managed intensively especially under dusty conditions (Davies, 1985; Bruere *et al*, 2002; Rahal *et al*, 2014). This is an area which requires further investigation; given that it is suggested that respiratory disease of sheep may be a significant cause of loss for the industry (University of Sydney, 2008). There is potential for infection leading to death or ill health especially during dry summer conditions; the effects of respiratory infections will be exacerbated in high environmental heat when sheep need to pant to relieve heat load (Rahal *et al*, 2014). High heat load is reported as a concern in the live export industry, when sheep travel through hot, humid conditions and in destination feedlots (Stockman *et al*, 2011), and the impact of respiratory infections in these animals should be assessed.

5.4 Hormonal and physiological measures on inappetent sheep

There was some correlation between time spent at the feed troughs and entry, exit and change in BCS, but not sufficiently strong that recommendations could be made regarding an optimum BCS for the process. Sheep with a higher BCS spent less total time at the feed troughs, perhaps indicating that the thinner animals had a higher drive to eat. There was no indication of the previously reported issues whereby very fat sheep did not eat and

developed a fatty liver/ketosis syndrome. Thus, it was not considered feasible or useful to remove the small number of sheep that were not eating from the large group for further study. Unpublished data from a previous study (Bowen et al. 2006) investigating the relationship between blood hormone concentrations at entry to a feedlot and the subsequent eating by more than 500 sheep did not show any predictive patterns of hormones, and this was not repeated (Stockman, 2006).

5.5 On farm backgrounding strategies to assist adjustment from pasture to pellet diet

Sheep that had access to pellets (trail fed in the paddock) for three days before departure to the feedlot were compared with sheep that had access to hay (in the paddock) one day before departure, and a control group which did not receive any supplementary feed. Sheep that had prior access to pellets spent less time spent at the feed troughs on the first day in the feedlot, spent less total and average time at the feed troughs, and visited the feed troughs less times than sheep in the hay or control groups. These results differ from previous studies, where exposure to pellets before feedlotting resulted in a greater number of sheep feeding at the feedlot than those that were not give prior supplementation with pellets (McDonald et al. 1988a).

The sheep in this backgrounding experiment spent considerably longer at the feed troughs each day than the sheep monitored in the earlier experiments without any interventions (an average over 48 minutes extra per day), and a high percentage in all groups attended the feed troughs from day 1 in the shed. These results may indicate prior experience with supplementary feed, which hastened acceptance of the pellets once at the feedlot, although the farmer apparently had not previously provided pellets on farm. Other on-farm factors may also influence acceptance of the diet and situation, such as selection for sheep of calm temperament (Bickell et al. 2008), prior handling and management that result in animals with confidence to readily adapt to novel situations (Keogh & Lynch 1984) and methods of handling and transport to the feedlot that limit stressors (Arnold and Charlick 1978; Cockram et al. 2004) and therefore reduce adverse responses to novelty.

5.6 Feedlot strategies to assist adjustment from pasture to pellet diet

The interventions were designed around what were seen as practical options for the industry. It was apparent that the feedlots were receiving sheep from a range of sources, the animals having had a diversity of background and management before they arrived at the feedlot. Therefore, interventions that could be applied as a blanket treatment to all animals at the feedlot were seen as possibilities. Two main areas were addressed: interventions to increase the percentage of sheep eating for an acceptable period as soon as possible after entering the feedlot (the initial “slope” of increase over the first five days), and interventions to increase the overall percentage of sheep eating for an acceptable period while in the feedlot (the “gap” up to 100% eating).

Feedlot staff may choose to accommodate sheep outside the sheds with access to hay as well as pellets for a day or two before the sheep are moved into the shed, with the thought that paddocks and hay are more familiar for the sheep and therefore the animals might more

readily accept the diet and conditions and therefore eat better. The shed environment is seen as more aversive and stressful, because it is unfamiliar to most sheep. There was no indication in the experiments conducted in August or December 2013 that this was the case. In August, by day 2 of the experiment, when all sheep were housed in the shed, the groups were spending the same amount of time at the feed troughs, and there was no difference in the percentage of sheep classed as inappetent between the groups. However, there were more *Paddock Hay Pellets* sheep (those that spent the first night in a paddock with access to hay and pellets) classed as inappetent on day 4, and from day 6 there were mortalities in that group, most due to salmonellosis/enteritis. As discussed above, these sheep may have been exposed to a higher *Salmonella* load in that initial period compared to the other groups, and developed disease, even though the appearance of clinical signs and mortality occurred slightly later than the usual incubation period of 1-4 days after exposure (House and Barnes, 2009). Deaths in that group continued when the feedlot staff chose to remove the animals from the shed to another outside paddock, on the assumption that the sheep would be better outside with access to pasture, and there were also several deaths from the Shed group when they were moved outside. The sheep in this experiment were also removed for shearing during the initial period and there were issues with the recording system so that some days' data was incomplete. The average amount of time spent at the feed troughs per day for these sheep was considerably less than for the average from the initial 8206 sheep monitored; the daily average time for the August 2013 sheep was just over one hour per day. It is not known why these sheep were not feeding for as long as other groups, but given their subsequent poor performance and high mortality, it is suggested that these may have been a group at high risk of health issues. Therefore, while it is difficult to detect and remove individuals who are inappetent, a general impression of feeding time may be possible for a group, that could then lead to other interventions. In this case, it is not known whether removing the sheep contributed to the continued mortality, or whether, having been exposed to *Salmonella*, the ensuing mortalities were inevitable.

In December 2013 there was again no indication that housing the sheep outside overnight on pellets and/or hay would improve acceptance of the pelletised diet in the shed. On the day they entered the shed the *Paddock Hay Pellet* group had a similar percentage of inappetent animals to the *Shed Pellet* group; it was not possible to monitor how many animals were eating for how long outside the shed. However, the *Paddock Hay Pellet* group did not perform better than *Shed Pellet* group, while the *Paddock Pellet* group performed worse in that they were delayed in having less than 90% inappetent sheep, and throughout the time in the shed there remained a higher percentage of those animals classed as inappetent. There were few deaths in this experiment, and the death rate was not different between the groups. The different mortality rate between August and December experiments may be related to an expected higher incidence of salmonellosis in the wetter times of year in WA (More 2002). That review found outbreaks appeared to be more common between the autumn break and early summer, and the risk of salmonellosis was substantially higher if animals arrived at the feedlot when the weather was cold, windy and wet.

However, the December 2013 cohort grew slowly and was considered by feedlot staff to be poorly performing overall, Several animals from each group were removed as unfit to transport. No further diagnosis was made as to the cause of poor performance.

The conclusion from these two experiments was that there was no benefit to housing sheep outside the sheds for a short period of a day before entering the shed. Housing in raised

sheds, where the animals have limited contact with faecal material and are dry and protected undercover, may limit exposure to *Salmonella*, and the other treatments did not hasten feed acceptance or increase the number spending an acceptable period at the feed troughs.

5.7 Additives to increase uptake and consumption of pellet diet

The addition of oats over the pellets did not result in any difference in uptake compared to sheep that were fed pellets alone.

The provision of chaff had some apparent effect as an attractant to the feed troughs, resulting in a change in the pattern of feeding. Sheep which were provided with chaff spread once per day over the pellets in the feed trough visited the feed troughs more often per day, but for shorter visits, than those given only pellets. In one experiment the mean time per day and total time at the feed troughs was greater for those provided with chaff, while in a second experiment there was no difference in mean time per day or mean total time at the feed troughs. In the first experiment, provision of chaff did not hasten acceptance nor alter the percentage inappetent after 6 days. In the second experiment, provision of chaff did apparently hasten acceptance of the feed, with less sheep in the chaff group classed as inappetent on day 1, compared to those given only pellets.

These findings confirm those of McDonald *et al* (1988b) who demonstrated feed intake benefits and improved patterns of intake in feedlot sheep when either oaten or lucerne chaff was added to a pelleted feed.

6 Conclusions/Recommendations

The use of RFID detecting technology was successful in detecting sheep at the feed and water troughs, with the tags confirmed as present and working using the portable RFID reader at exit from the feedlot. The system was used to quantify the amount of time individual sheep spent at the feed and water troughs within a determined time frame. The correlation of BCS and total time at feed trough provided biological evidence that the tracking system successfully detected those that were not feeding.

The sheep spent on average over 1.5 hours total time at the feed troughs per day, and the sheep that lived spent more than an hour longer per day at the feed troughs, corrected for day of death. Sheep were defined as inappetent if they spent less time than the mean daily average minus 2 standard deviations, ie less than 28 m 5 s at the feed troughs. Many animals which died fitted into that definition, and the mean daily average time for sheep which died was less than that, confirming that a short time feeding was associated with mortality. Whether inappetence was cause of death alone or the anorexic effect of fatal disease could not be distinguished.

Using this definition of inappetence, the daily variation in time at feed troughs plateaued by day 6 in the feedlot, by which time 95% of sheep were spending more than the minimum time at the feed troughs. There was a further slight decrease in percentage of sheep defined as inappetent for mobs monitored for longer periods, but even for those groups, there was at least 2% defined as inappetent on any day. It was not always the same sheep that fed only minimally each day.

The study indicated that there is little economic advantage to be had in early detection and removal of those animals which are not going to the feed troughs, because the mortality was relatively low, and because not eating or eating for short periods of time on any day was not necessarily predictive of death, i.e. the feeding pattern of those that died was not consistently different from those that did not die. Removing sheep that had not attended the feed trough on any given day would mean disrupting the large group, with many misclassifications as to “at risk” sheep.

It is possible that this system can be used in conjunction with other projects that require information on drinking and feeding behaviour of sheep, such as delivery of water-based medications.

There were apparent differences in period of acceptance and percentage feeding adequately between different cohorts of sheep at different times of the year, but this could not be statistically tested. Different feeding preparation immediately before trucking and feedlotting did not affect feeding performance at the feedlot.

The main cause of mortality at this feedlot was diagnosed as salmonella/inanition, especially in winter. This corroborated previous work that indicates that the two syndromes are linked (Moore 2002). Feeding patterns associated with development of salmonellosis include intermittent or interrupted feeding after exposure to salmonella.

Feeding interventions assessed in this project did not markedly hasten feed acceptance or increase the number spending an acceptable period at the feed troughs. The only strategy which had some effect was the provision of chaff on the pellets, and this is used in industry with animals considered of concern; the data continues to support the practice. The risk of salmonellosis should be considered with any change in management strategy. There was no gain to housing sheep outside the sheds for a short period of a day before entering the shed. Housing in raised sheds, where the animals have limited contact with faecal material and are dry undercover, may protect them from exposure to *Salmonella*.

Other means of reducing contamination will be important in limiting *Salmonella* infection, for instance with all in/all out management, and not running newly received sheep through the same areas as those that leave (circular flow to limit exposure of new sheep to organisms). The continued development of a vaccine against salmonellosis is very likely to have an important impact on reducing mortality in similar situations to those tested here.

The contribution of pneumonia to feedlot mortality and morbidity is not commonly associated with pre-embarkation feedlots and shipping; however, it is an area which requires further investigation. Death rates from pleuropneumonia were found in one group during summer in this study and it has been suggested that respiratory disease of sheep may be a significant cause of loss for the industry (University of Sydney, 2008). There is potential for infection leading to death or ill health especially during dry summer conditions and the effects of respiratory infections will be exacerbated during high heat load when sheep need to pant (Rahal *et al*, 2014). High heat load is reported as a concern in the live export industry, when sheep travel through hot, humid conditions and in destination feedlots (Stockman *et al*, 2011), and the impact of respiratory infections in these animals should be assessed.

7 Key Messages

- The sheep spent on average over 1.5 hours total time at the feed troughs per day, while those defined as inappetent spent less than half an hour per day at the feed trough.
- Sheep in intensive feeding systems spending less than half an hour at the feed trough are therefore considered not to be feeding adequately. However, it may not be easy or worthwhile to remove these inappetent sheep, because it is not always the same sheep that do not eat on any day, and the disruption of the whole group may be counterproductive. Remote/non-intrusive detection and removal methods such as electronic detection and automatic drafting may allow removal of animals that are not feeding for 4-5 days, which are the animals most “at risk”.
- It took until day 6 in the feedlot for more than 95% of sheep to be spending more than the minimum time at the feed trough per day. This supports recommendations that require sheep to have 5 clear days at the registered premises before export (S3.8 a (i) ASEL 2011). After the 3 clear days at the registered premises referred to in subsequent sections of Standard 3.8 there may be over 85% of sheep spending more than the minimum time at the feed trough per day, but the animals may still be establishing normal patterns of eating pellets.
- Enteritis, mostly associated with isolation of *Salmonella spp*, combined with inappetence/inanition, was diagnosed as the most common cause of death. Therefore control of salmonellosis appears key to reducing mortality. Any feeding interventions must limit exposure to *Salmonella* e.g. from environmental contamination. Inconsistency of feed intake appeared important in the development of salmonellosis, so maintaining consistent feed intake is important in limiting disease.
- No feeding interventions tested at the feedlot increased the percentage of sheep feeding adequately after five to six days. In one experiment, provision of chaff spread over the pellets apparently hastened the acceptance of the feed, compared to pellets alone. Therefore there are no standard recommendations for feedlot feeding at introduction. It should be noted that astute stockmen at a feedlot may consider groups more or less of concern at entry, and adapt feeding accordingly. The use of chaff spread on the pellets in the troughs remains the only intervention which has shown any alteration in feeding pattern.
- There was variation in the eating patterns and mortality of sheep from different groups, which may reflect different preparation and management. These sheep factors prior to feedlotting should continue to be investigated, with the prospect of rewarding producers whose animals perform well.
- **Best practice guidelines for pre-embarkation treatment of sheep to minimise the incidence of inanition/salmonella**

On farm preparation	<p>Ensure the sheep are up to date with health care:</p> <ul style="list-style-type: none"> -Vaccination for Scabby mouth -Vaccination for Clostridial diseases (particularly pulpy kidney/enterotoxaemia) -Internal parasite control -Recent Vitamin E supplementation, especially for young sheep coming off dry pastures
Feeding and management	<p>Exposure of young stock to supplementary feeding, as lambs with their mothers, and/or as older sheep. The supplementary feeding should provide pellets or grain or mixed ration, trail fed or in troughs, to expose the animals to novel feeds and different methods of supply. Recommendations for low stress stock handling during yarding and trucking should be followed.</p>
Conformation with ASEL V2.3 guidelines:	<p>Age, class, weight and condition score of animals to meet guidelines and specific consignment requirements.</p> <p>Curfew not more than 12 hours off green feed, may have access to dry feed and to water. Consideration should be given to provision of hay overnight before trucking for animals coming off lush green feed, and for animals which may have extended periods (>12 hours) before accessing feed at the feedlot, so that they start the transport process with a rumen containing roughage.</p>
At the feedlot	<p>Limit the period for animals on the truck; this requires coordination of the trucking schedules with feedlot operations so that animals are not held overnight before unloading.</p>
<p>Low stress stock handling for unloading and moving the animals. Circular movement of stock is preferred, rather than back and forth (where incoming animals move over ground potentially contaminated by outgoing animals).</p>	
<p>Initial housing can be straight into the sheds, with the original cohort of animals, rather than mixing of animals. Subsequent moving and mixing may be required,</p>	

and may be less disruptive once the sheep have developed greater familiarity with the new premises.

Stock handlers can assess the groups through routine monitoring; aspects to note include how quickly the animals access the feed troughs after entry to the sheds, how many sheep go to the feed troughs or stay away, how quickly the sheep return to the feed troughs after disruption of the group, how confident or flighty the animals are with people moving around. Sheep which appear flighty, not confident, and not easily and quickly accessing the feed troughs should be considered at higher risk of inappetence.

Animals which appear not to be feeding well or which are noted from entry to be quiet or depressed can be supplemented immediately on entry with chaff (oaten or Lucerne) spread around on top of the pellets, and scattered nearby the troughs. This may enhance their initial approaches to the troughs. Daily application of chaff to the feed troughs for at least 2-3 days is recommended for such groups.

Paddock accommodation should be into clean, well drained paddocks, with feed supplied off the ground to limit contamination. In wet, cold weather it is preferable to house the sheep directly into clean, covered sheds.

Faecal consistency can be noted daily; loose faeces or diarrhoea is indicative of digestive upsets and enteric infections. Application of chaff to the feed troughs or provision of hay if logistically possible may assist in maintaining feeding and digestion.

Sheep should ideally be at the feedlot for at least 5 days for more than 95% of animals to be eating well.

For groups of animals which appear confident and eating well, shearing on any day of feedlotting may not interfere with their eating. Groups of animals which are not relaxed and confident and have more animals not accessing the feed troughs may develop disease if taken away from feed for shearing.

- **Recommendations for future work**

Continued development, testing and commercialisation of a vaccine against salmonellosis.

Investigation of on farm factors which develop robust resilient sheep, confident in novel situations.

Non-invasive behavioural and health assessment of groups of animals, to quickly identify cohorts at higher risk of inappetence and disease.

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9 Appendix

9.1 Statistical modelling to predict at-risk sheep

The time spent at the troughs per day by each individual, and their outcome as live or dead, was used in linear discriminant analysis to classify sheep as 'at-risk' or 'not-at-risk' based on a linear function incorporating previous days' feeding times.

The probabilities of assignment of individual sheep to the two groups in this modelling approach required decisions as to:

- the prior probabilities π and $1 - \pi$ of assignment to the at-risk and not-at-risk groups, respectively, and
- which days' feeding times were to be used in the linear discriminate analysis (LDA) to best classify sheep.

Specification of prior probabilities

In the absence of exogenous information pertaining to the likelihood of a sheep being at risk, the prior probabilities π and $1 - \pi$ are typically specified as the observed percentages falling into each group. For the collected data on 8,206 sheep, after the first day, these probabilities were 0.009 and 0.991 for the at-risk (75 dead) and not-at-risk (8,131 alive) groups, respectively.

When using these observed percentages as prior probabilities in LDAs after each of the first 7 d, the resulting classifications labelled all sheep as not at risk because of the rarity of death as well as the similarity in eating patterns exhibited by sheep that died and numerous sheep that did not die. Labelling all sheep as being not at risk leads to the highest rate of correct classification, but in this context it is far more important to correctly identify at-risk sheep than not-at-risk sheep.

To address this issue, a 'loss function' was specified to reflect the costs of incorrectly classifying members of one group as being in the other. Such costs may be quantifiable costs such as economic loss due to death or more expensive feed or examination methods for sheep deemed to be at risk, or they can alternatively or additionally reflect qualitative costs such as perceived effects on animal welfare. When only two groupings are under consideration, only the relative costs matter (Huberty and Olejnik, 2006, p. 350), so, if C_1 denotes the cost associated with incorrectly classifying an at-risk sheep as being not at risk and C_2 denotes the cost associated with incorrectly classifying a not-at-risk sheep as being at risk, then the ratio C_1/C_2 is sufficient. Further, incorporation of these costs can be accomplished through adjustment of the prior probabilities π and $1 - \pi$, producing new priors

$\pi^* = \frac{\frac{C_1}{C_2}\pi}{\frac{C_1}{C_2}\pi + (1-\pi)}$ and $1 - \pi^*$. Thus, the costs modify the prior probabilities of assignment to the

groups by giving greater preference to the group with higher cost associated with misspecification. If the cost associated with incorrectly labelling an at-risk sheep as being not at risk is significantly higher than the reverse, the sensitivity of the LDA is increased (at the expense of the specificity).

Variable selection

The preferred methods for variable selection for LDA for predictive purposes (“predictive discriminant analysis”) are variants of the method of Habbema and Hermans (1977), where all possible subsets of variables are considered. Using leave-one-out cross validation, data for all the other sheep were used as a training set to fit the model, and the sheep under consideration served as the validation set.

The optimal subset of variables was selected according to two criteria:

1. the subset of variables that minimised the loss function, and
2. the subset of variables that maximised specificity (i.e. rate of correctly classifying not-at-risk sheep) while guaranteeing a desired sensitivity (i.e. rate of correctly detecting at-risk sheep).

The optimal subset of variables is dependent on the choice of prior probabilities, as previously highlighted regarding the impact of choice of priors on classifications. This chosen approach simultaneously considers all subsets of previous days’ feeding times as well as a range of prior probabilities to select the subset of variables and prior probabilities that are optimal for the given criterion. To implement the approach, we used the R statistical language (R Core Team, 2013) and the MASS package (Venables and Ripley, 2002).

Linear discriminate analysis (LDA)

Results for LDAs for 1 to 7 d elapsed are presented, highlighting the relevant previous days’ feeding times and priors that should be used to, in the one case, minimize the loss function and, in the other case, maximise specificity while maintaining a particular sensitivity.

Variable selection and prior selection for loss functions

The LDA was considered for all possible subsets of feeding times for 7 d of feeding, and for a range of priors, starting with priors given by the observed percentages in each group and then increasing the relative weight of π , the prior probability of being at risk, by factors of 2 to 500. For each possible combination of variable subsets and priors, the resulting LDA classifications obtained through leave-one-out cross validation were compared to the actual groups to which the sheep belonged, to calculate the number of misclassifications for each group and resulting total cost based on C_1 and C_2 . The subset and prior which produce the minimum loss is the preferred subset and prior.

Table 4 shows the preferred subset of previous days’ feeding times and prior probability π that minimise the loss function after a certain number of days have elapsed. In the cases where 1, 2, 6, and 7 d have elapsed, only the previous day’s feeding time was useful in predicting whether or not a sheep was at risk. The comparative boxplots (Fig. 8) illustrate that the distinction between at-risk sheep and not-at-risk sheep in terms of feeding times is readily apparent in days 6 and 7. Feeding time on day 2 is important in predictions after 2, 3, 4, and 5 d have elapsed, and day 5 is important after 5 d have elapsed.

Variable selection and prior selection for a desired sensitivity

When the criterion is changed to maximising specificity for a Type I error rate of $\alpha = 0.05$ (i.e. the LDA should be able to detect at least 95% of at-risk sheep), the variable selection algorithm finds that the first two days of feeding times are sufficient to guarantee a Type I error rate of $\alpha = 0.05$ after days 2 to 5 (Table A1).

The expected minimum loss decreases as more days elapse, and the expected number of misclassifications of not-at-risk sheep also decreases. From days 1 to 7, there is a minuscule reduction from 3 misclassified at-risk sheep to 2; the number of misclassifications of not-at-risk sheep drops from 7,853 to 3,327 sheep from days 1 to 7 with the approach better able to correctly identify sheep that are not at risk.

Table A1. Subsets of previous days' feeding times and priors that maximise specificity for a desired sensitivity for a given number of days elapsed. The total number of misclassifications of not-at-risk sheep is also presented.

Days Elapsed	Subset of Days	Prior (π)	Misclassifications
1	1	0.7326	7,853
2	1, 2	0.7596	6,534
3	1, 2	0.7596	6,534
4	1, 2	0.7596	6,534
5	1, 2	0.7596	6,534
6	2, 3, 5, 6	0.7724	4,124
7	2, 5, 6, 7	0.7656	3,327

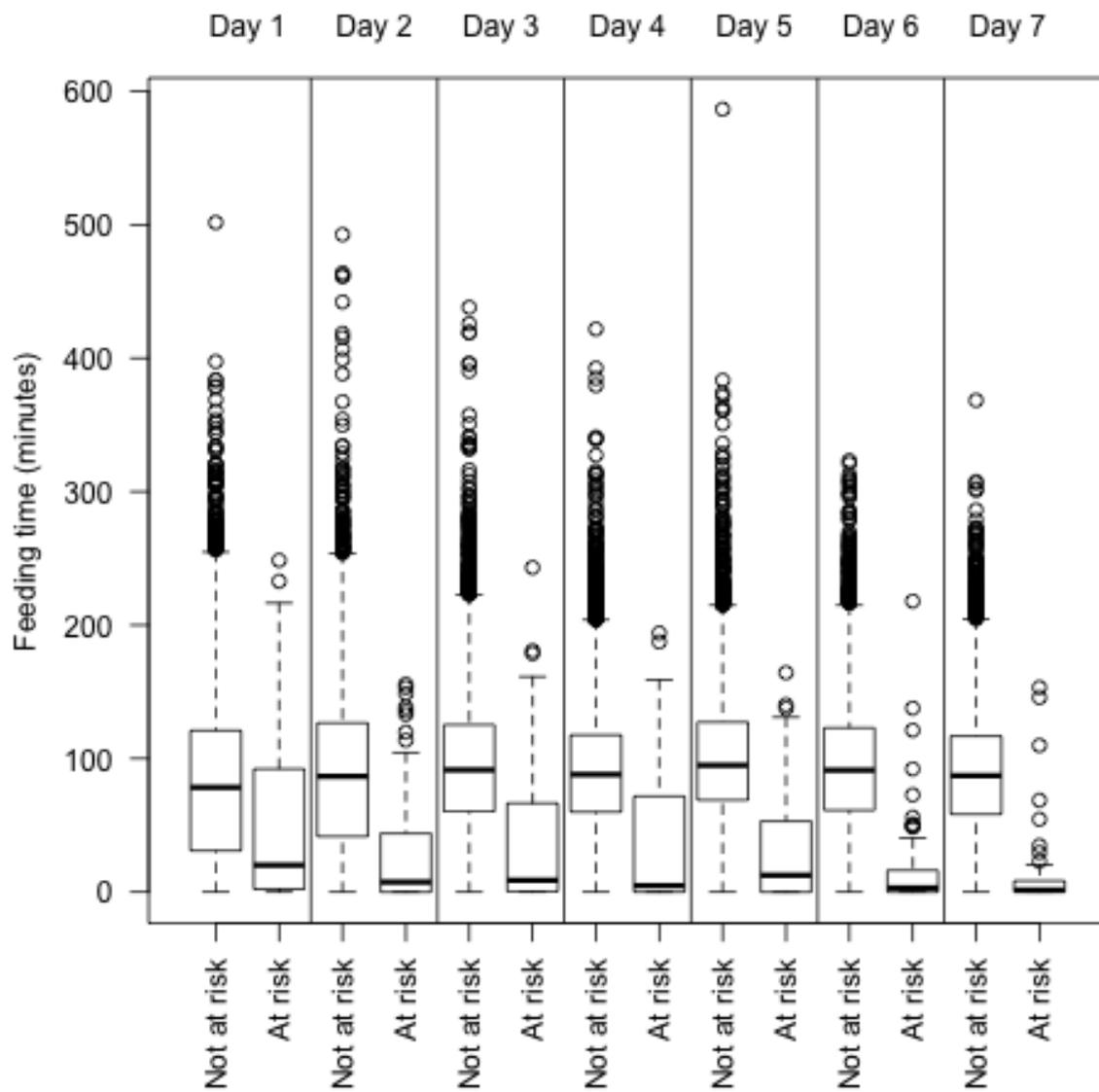


Figure A1. Feeding times for at-risk (i.e. subsequently died) and not-at-risk sheep for days 1 to 7.