Development and Validation of Animal-based Welfare Indicators for a Precision Livestock Farming (PLF) Approach to Small Ruminant Welfare Management

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Authorship Declaration

I declare that I am the sole author of this thesis and that it has not been submitted, in whole or in part, in any previous application for a degree. All writing and analyses are my own work, conducted with the help of my supervisors. Cathy Dwyer, Fiona Kenyon, Emma Baxter and Jessica Martin provided guidance on methodology, analyses and writing.

I collected data for Chapter 2 from a trial organised by another PhD student, Naomi Booth. The only exception to this were the feed intake and faecal egg count data, which Naomi was collecting for her research and shared with me. I contributed to the daily running of this indoor trial alongside Naomi, her supervisors and SRUC technicians. All statistical analyses were completed by me.

Data for Chapter 3 and 4 were comprised of two field experiments. I carried out the fieldwork with assistance from technicians, post-doc Dr. Heather McDougall, and a team of researchers from the Moredun Research Institute as the trials were simultaneously being used as pilots for the wider TechCare EU Horizon 2020 project. I collected all behavioural and welfare data and contributed to the faecal egg counts. SRUC research technicians Jo Donbavand and Marianne Farish collected behavioural data for three weeks in 2022 when I was called home for an emergency. Data from PLF were jointly collected by Heather and me. All statistical analyses were completed by me.

Data for Chapter 5 were collected by TechCare-funded PhD student, Aimee Walker, for one of her experiments and shared with me. I contributed to the methodology planning for this data collection. All statistical analyses were completed by me.

Data for Chapter 6 were collected by me after designing the interviews with the help of SRUC Research Fellow Lesley Jessiman. All analysis was conducted by me, with guidance from Lesley.

All help received during the project has been truly valued.

Michelle Reeves

Abstract

To improve animal welfare management on sheep farms, it must first be measured through reliable welfare indicators. In extensive management systems, gathering welfare information is labour intensive and time-consuming. In a rural environment where the labour force is dwindling and the average age of farmers is climbing, technology is often declared to be an all-encompassing solution. Precision livestock farming (PLF) is defined as managing individual animals by continuous real-time monitoring of health, welfare, production, reproduction, and environmental impact, essentially every aspect of a farmed animal's life. It utilises all types of technology to allow farmers to collect information efficiently and accurately about individual animals. In sheep farming, commercialised PLF tools are rare. However, sheep behaviour has been reported to change when welfare challenges occur. For example, grazing patterns are altered by gastrointestinal parasitism and lameness affects the smoothness of movements. Therein lies an opportunity to use technology to monitor sheep behaviour and identify when welfare challenges occur in extensively raised sheep. However, PLF development and roll-out face challenges such as high costs, rare validation and the ethical questions surrounding increased automation in animal farming.

This thesis is composed of a series of experiments on domestic sheep (*Ovis aries*) to test the accuracy of potential welfare indicators and the ability of two PLF tools to record them. I hypothesised that accelerometers and Bluetooth beacons would be able to measure behavioural changes in sheep experiencing lameness, gastrointestinal parasitism and mastitis, as these are some of the most important welfare challenges faced by extensively managed sheep. A pilot trial was undertaken indoors to examine if any behavioural changes occurred during gastrointestinal parasitism that could eventually be monitored by PLF approaches (Chapter 2). This experiment took advantage of a large parasitological trial, organised for other purposes, to look for behavioural differences between lambs parasitised with *Teladorsagia circumcincta* and healthy lambs. Behaviour was monitored through video recordings that were scan sampled and behaviour sampled, and in-person using Qualitative Behaviour Assessment (QBA). Lambs were separated into three treatment groups: 1) ad-lib fed controls (AC), 2) restricted-fed controls (RC) and 3) ad-lib fed parasitised lambs (AP). Parasitised lambs were found to be more likely to be standing inactive than AC lambs

and less likely to be eating than RC lambs over the first 3 weeks of infection. Parasitised lambs had higher loadings on the QBA dimension describing fear and anxiety compared to RC lambs. These results were interpreted as reflecting the discomfort caused by abomasal damage inflicted by *T.circumcincta* larvae and the expected parasite-induced anorexia. Chapter 2 also offered novel evidence that not only did parasitism negatively impact lamb health but it also affected their mental state by increasing levels of fear and anxiety.

Chapter 3 describes trials occurring over two grazing seasons where 56 ewes and 112 lambs faced natural infection with lameness, mastitis, and gastrointestinal parasites. Daily scan sampling, weekly QBA and fortnightly welfare assessments monitored their behaviour and welfare. Welfare assessments consisted of recording weights, dag scores, fleece, breathing and injury scores for all sheep, and additional body condition scoring and dentition scores for ewes. Lameness scoring, faecal egg counts (FEC), and mastitis scoring by udder palpation for ewes monitored the diseases of interest during both years, with somatic cell counts (SCC) of ewe milk samples being added in the second year of the trial. Generalised linear mixed models (GLMM) were used to analyse the relationships between behaviour and welfare indicators. Grazing behaviour in lambs was significantly associated with lamb lameness and parasitism. There was a significant interaction between lamb lameness score and strongyle FEC that affected their locomotion and lying behaviour. Nematodirus FEC had a significant impact on lamb lying, standing and play. Ewe lameness was associated with lying behaviour. Chapter 3 concluded that ewe and lamb parasitism and lameness had the potential to be identified through behavioural indicators.

Chapter 4 aimed to validate the use of AX3 accelerometers (Axivity Ltd., Newcastle-upon-Tyne, UK) to categorise ewe and lamb behaviour. To do this, 6 ewes and 6 lambs were observed using 20-minute focal samples on 4 days. Their recorded behaviours were compared to the AX3 outputs. This chapter also tested whether wearing a collar containing the AX3 had any impact on sheep behaviour or welfare. This was done using data from the trials in Chapter 3 since half of the animals were wearing collars containing technology while the other half acted as a control group, not wearing collars. Results showed that ewe rumination was less likely to be observed when they were wearing collars. Ewes and lambs had a higher probability of presence of strongyle eggs in faecal samples when they were not wearing collars. This implies that grazing behaviour may have differed between sheep with and without collars, leading to increased exposure to strongyle larvae for the “no collar” control group. The AX3 validation was attempted using a series of statistical methods. Very low levels of variation in the accelerometer data made comparison of behaviours challenging. However, k-means clustering partially categorised some behaviours, such as grazing and standing. The validation was not entirely successful, but it led to the conclusion that unlabelled machine learning techniques may be able to help complete this validation with more variable data and purpose-built algorithms. Further work is required to clarify the findings in this chapter suggesting collars could impact rumination and grazing behaviour.

A second PLF tool was tested in Chapter 5: Bluetooth Light Energy (BLE) beacons (Feasycom, Shenzhen, China). This study tested their ability to monitor ewe-lamb distance as an indicator of welfare. Collars containing the BLE beacons were put on lambs as they were born, while the ewes wore purpose-built readers called Wearable Integrated Sensor Platform (WISP) readers on collars. The WISP readers transmitted a Received Signal Strength Indicator (RSSI) via a low-power wide-area network (LPWAN) gateway every 5 minutes for the 16 beacons closest to it. RSSI was converted into distance in metres and the data was filtered so that only the observations concerning the distance between each ewe and her offspring would remain. Weekly welfare assessments were carried out in person on all animals for 6 weeks after the start of lambing. These assigned binary lameness, fleece and dag scores to every animal (0-absence of welfare issue/1-presence of welfare issue). All lambs had lameness, fleece and dag scores of 0 for the duration of the study, rendering it impossible to draw any conclusions about ewe-lamb distance as an indicator of lamb welfare. However, ewes with a lameness score or a fleece score of 1 had shorter ewe-lamb distances, meaning their lambs remained closer to them than lambs who had dams with lameness or fleece scores of 0. Chapter 5 concluded that ewe-lamb distance is associated with ewe welfare indicators and can be measured by PLF.

Chapter 6 describes the findings from explorative semi-structured interviews with Norwegian sheep farmers about their use of PLF technology. Norway has a high rate of PLF adoption in livestock farming compared to other European countries, and therefore offered the opportunity to study the motivations and perspectives of producers currently using technology. Twenty-four farmers from three regions in

Norway who use a form of technology on their sheep farms were interviewed. The interview was designed to understand what drove their initial adoption of PLF, why they continue to use it or not, and their vision of the future with PLF in sheep farming. Reflexive thematic analysis identified five main themes from the farmers' responses: Resources and Savings, Control and Decision-making, Governmental Influences and Pressures, Out with the Old and In with the New, and Curiosity and Excitement. Many decisions were driven by farm economics, where PLF improved a costly process or saved the farmers time. Several farmers referred to the increased amount of control that PLF offered them over their flock. The government was seen as a source of both support and hindrance to farmers using PLF. Participants expressed a perceived incompatibility between PLF and older users, although this was not reflected in their reality as many older farmers interviewed for this study invested heavily in technology. And finally, many farmers simply found the extra information they gained was fun, interesting, and satisfying. The farmer motivations identified in this chapter have implications for our understanding of how and why PLF is applied on farm and could inform the development of future technologies.

These findings suggest that behaviours could be used to monitor the welfare of sheep in extensive management systems. Because in-person monitoring is time-consuming, PLF tools have been found to have potential to monitor the changes in behaviour. However, this thesis also reported the importance and challenges of validating such technology. This highlights the need for robust, independent validation studies to support the growing interest in PLF for livestock. The findings related to behavioural indicators and farmer motivations around PLF have implications for the future development of PLF tools for welfare monitoring.

Lay Summary

Improving animal welfare management on sheep farms relies on having accurate tools to measure it. In hill farming systems, recording sheep welfare using traditional assessment techniques requires handling the animals, is labour intensive and time-consuming. Precision livestock farming (PLF) is defined as managing individual animals by monitoring health, welfare, production, and reproduction, essentially every aspect of a farmed animal's life. It utilises all types of technology to allow farmers to collect information efficiently and accurately about individual animals to support their decision-making. In sheep farming, commercialised PLF tools are rare. However, research shows that sheep change their behaviour when faced with welfare challenges. For example, grazing patterns are altered by gastrointestinal parasitism and lameness affects the smoothness of their movements. This creates an opportunity to use PLF to monitor sheep behaviour and identify when welfare challenges occur. However, the appropriate behaviours to record using technology must first be identified and their relationship with welfare must be understood.

This thesis tested the accuracy of potential welfare indicators in sheep and the ability of two PLF tools to record them. It was expected that two types of technology (accelerometers and Bluetooth Low Energy (BLE) beacons) would be able to measure behavioural changes in sheep facing lameness, gastrointestinal parasitism, and mastitis, as these are some of the most important welfare challenges faced by hill sheep. Using quantitative and qualitative methods, the first experiment examined if any behavioural changes occurred during gastrointestinal parasitism in lambs that could eventually be monitored by PLF technologies. Parasitised lambs were more likely to be standing still and less likely to be eating than non-parasitised lambs during the first 3 weeks of infection. Parasitised lambs also behaved more fearfully than healthy lambs, suggesting that their behaviour and welfare were affected by the parasite infection.

A large experiment was then created to analyse the relationships between behaviour and welfare challenges. It suggested that several lamb behaviours were affected by lameness and parasitism, including grazing, standing, lying, walking, and playing, meaning these behaviours had the potential to act as indicators of welfare challenges. Ewe lying behaviour was associated with lameness score. The data from this trial was

also used to test whether wearing a collar containing technology had any impact on sheep behaviour or welfare. Results showed that ewes were less likely to ruminate when they were wearing collars and ewes and lambs were more likely to be infected with parasites when they were not wearing collars. This implies that grazing behaviour may have been affected by collars, since not wearing a collar seems to have led sheep to be more exposed to parasite larvae on grass.

Accelerometers, or activity monitors, were then validated to test if they could identify the behaviour of the sheep wearing it. The accelerometers could identify grazing and standing in ewes and lambs, but not any other behaviours, meaning further validation studies are required. The following study tested if the distance between a ewe and her lamb measured by BLE beacons could be used as a welfare indicator. It found that lame ewes and ewes with missing patches of fleece, which can be a sign of stress or health issues, had shorter ewe-lamb distances. Their lambs remained closer to them than lambs who had dams with no lameness or fleece issues. This study concluded that ewe-lamb distance is associated with ewe welfare. This means there is potential for PLF technology to measure this kind of welfare information.

The final study was a series of interviews with Norwegian sheep farmers who currently use PLF to find out what motivated them to use it and what their preferences were regarding PLF in general. The interviews revealed that farmers used PLF because they felt it would save them time and money, it would increase their level of control over their flock, and because they were curious to learn more detailed information about their animals. They were positively and negatively influenced by the government, and they felt that younger people were more interested in technology.

Overall, this thesis showed that behaviours like grazing and standing have the potential to be used as sheep welfare indicators and that PLF can monitor the changes in these behaviours. However, it also reported the importance and challenges of validating such technology. The findings related to behavioural indicators and farmer motivations around PLF could help guide the creation of future PLF tools for welfare monitoring.

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List of Abbreviations

Abbreviation	Long form	Abbreviation	Long form
AC	Ad-lib fed control	GNSS	Global Navigation Satellite System
AHDB	Agriculture and Horticulture Development Board	GPS	Global Positioning System
AP	Ad-lib fed parasitised	HERC	Human Ethical Review Committee
AWC	Animal Welfare Committee	HMM	Hidden Markov Model
AWERB	Animal Welfare Ethical Review Board	HP	High Parasitism
AWIN	Animal Welfare Indicators	kg	kilogram
AX3	Accelerometer model	LP	Low Parasitism
BCS	Body Condition Score	MBS	Maternal Behaviour Score
BLE	Bluetooth Low Energy	ml	millilitre
C1 - 7	Cluster 1-7	MMI	Mean Motion Index
CMT	California Mastitis Test	PC1	Principal Component 1
CSV	Comma Separated Values	PC2	Principal Component 2
DEFRA	Department for Environment, Food and Rural Affairs	PC3	Principal Component 3
DOI	Day of Infection	PEOU	Perceived Ease of Use
EFSA	European Food Safety Authority	PLF	Precision Livestock Farming
EID	Electronic Identification	PU	Perceived Use
epg	eggs per gram	QBA	Qualitative Behaviour Assessment
FAWC	Farm Animal Welfare Committee	RAM	Random Access Memory
FEC	Faecal Egg Count	RC	Restricted-fed Control
FSS	Faecal Soiling Score	RFID	Radio Frequency Identification
g	gram	SCC	Somatic Cell Count
GAMM	Generalised Additive Mixed Model	SCOPS	Sustainable Control of Parasites
GI	Gastrointestinal	SHAWG	Sheep Health and Welfare Group
GLMM	Generalised Linear Mixed Model	TST	Targeted Selective Testing

TAM	Technology Acceptance Model	UHF	Ultra High Frequency
TPC	Theory of Planned Behaviour	UK	United Kingdom
TRI	Technology Readiness Index		

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Publications from the Thesis

Chapter 2 of this thesis has been published in Applied Animal Behaviour Science, https://doi.org/10.1016/j.applanim.2024.106323. This chapter was accepted for publication between the time of submission of this thesis and the examination. Therefore, the originally submitted manuscript is presented in the body of this thesis and the final published version can be found in Appendix A.

Chapter 1. General Introduction

Extensive meat sheep management is a system where the stockperson is almost never with the sheep, which are kept on unfenced pastures with no housing, while semi-extensive systems are those where the stockperson is not continuously with the sheep, who are kept on often improved, fenced pastures and may be housed for lambing (EFSA, 2014). Throughout this thesis, the term « extensive » will be used to refer to British extensive and semi-extensive systems. There is a generally held belief that extensive environments automatically deliver better welfare than indoor management systems (Temple & Manteca, 2020). These types of systems are perceived by consumers as providing good animal health and high welfare as animals' behavioural needs are more easily met than in intensive indoor systems (Rioja-Lang et al., 2020; Spigarelli et al., 2020; Verbeke et al., 2008). However, a changing environment and rare opportunities for monitoring, diagnosis and treatment lead to unique welfare risks in extensive conditions, typically centred around disease and injury (Munoz et al., 2018; Richmond et al., 2017; Rioja-Lang et al., 2020; Spigarelli et al., 2020). A 2014 European Food Safety Authority (EFSA) report identified the principal welfare challenges for sheep in European extensive production systems. These included prolonged hunger, pain due to management procedures, lameness, parasitism, mastitis and neonatal disorders (EFSA, 2014). Despite extensive systems being highly valued by consumers for their "naturalness," the potential for prolonged hunger is often forgotten (Goddard et al., 2006). In the UK, lambs are still often subjected to painful yet routine management practices such as tail docking and castration for male lambs without analgesia (Dwyer, 2008). Lameness is the main symptom of limb injury, most frequently footrot and scald, also called interdigital dermatitis (Winter et al., 2015). Recent farmer surveys suggest that on average 3.2% of ewes are lame on UK farms (Best et al., 2020). Internal parasites are concerning especially in areas with high rainfall (EFSA, 2014), such as the UK. Though mastitis is often researched as a dairy sheep disease, it can cause acute levels of pain in meat ewes, which goes on to impact the welfare of their lambs (Fthenakis & Jones, 1990). The EFSA report's claims are further supported by a large body of literature emphasizing the important effects of lamb mortality, dystocia, and other perinatal conditions on sheep welfare (Dwyer &

Lawrence, 2005; Matheson et al., 2011; Rooke et al., 2015; Smith, 1977). Researchers and producers alike have cited ectoparasites as a major health and welfare concern (Goddard et al., 2006; Morgan-Davies et al., 2006; Plant, 2006). Having identified these welfare risks in extensive systems, the challenge of monitoring them remains. Managing animals over difficult terrain can be impractical and expensive, especially as the skilled rural labour force dwindles (Goddard et al., 2006; Richmond et al., 2017). The lack of stockpeople itself has become a welfare concern (Rioja-Lang et al., 2020). Digital technologies have been proposed as a solution to this problem, helping farmers be efficient and profitable (Kaler & Ruston, 2019). This thesis will study lameness, gastrointestinal parasitism, and mastitis as examples of welfare risks for sheep in extensive and semi-extensive systems that have the potential to be managed through technology with Precision Livestock Farming (PLF). To be managed, they first need to be identified.

1.1 Welfare Assessments and Frameworks

Consumers show a continued concern about farm animal welfare (European Commission, 2023). This is reflected by the inclusion in 2016 of a recommendation entitled “Animal health and welfare” in the United Nations Committee on World Food Security’s “Proposed draft recommendations on sustainable agricultural development for food security and nutrition including the role of livestock” (United Nations, 2016). The International Finance Corporation (IFC) published a Good Practice Note in 2014 entitled “Improving Animal Welfare in Livestock Operations” (IFC, 2014). This societal evolution has led to an increased need for on-farm assessment of welfare standards for certification, farm assurance, and legal purposes (Phythian et al., 2011). These assessments can allow benchmarking and enable farmers to manage their animals’ welfare directly (Phythian et al., 2011). Their implementation requires the development of practical and scientifically validated methods (Mattiello et al., 2019; Winckler et al., 2003). To address this need, common welfare frameworks for stakeholders to work within have been established.

The Five Freedoms paradigm was the first to use subjective experience, health, and behaviour to describe the dimensions of animal welfare (Webster, 1994). It was first presented to the public in a press release from the Farm Animal Welfare Council in December 1979 (FAWC, 2012). Each freedom is accompanied by a provision, which describes related management and care instructions necessary for safeguarding and improving welfare (FAWC, 2012). The Five Freedoms are: 1) freedom from hunger and thirst by ready access to fresh water and a diet to maintain full health and vigour, 2) freedom from discomfort by providing an appropriate environment including shelter and a comfortable resting area, 3) freedom from pain, injury or disease by prevention or rapid diagnosis and treatment, 4) freedom to express normal behaviour by providing sufficient space, proper facilities and company of the animal's own kind, and 5) freedom from fear and distress by ensuring conditions and treatment which avoid mental suffering (FAWC, 2012). These are described as “ideal states rather than standards for acceptable animal welfare” (FAWC, 2012). Many early and current codes of practice reference the Five Freedoms, such as the Handbook of Laboratory Animal Management and Welfare, first published in 1998 or the Canadian Council on Animal Care’s “Guide to the Care and Use of Experimental Animals” from 1993 (CCAC, 1993; Wolfensohn & Lloyd, 2013). They often feature in policy statements and legislation, such as the UK Animal Welfare Act 2006 (Mellor, 2016), and have been adopted by international organisations such as the World Organisation for Animal Health (OIE, 2019). However, this framework does not differentiate between functional (e.g. malnutrition, disease) and affective (e.g. pain, fear) aspects of animal welfare (Mellor & Reid, 1994). Furthermore, by describing only ideal states, it does not enable assessors to determine whether an animal’s welfare is currently at an acceptable level, which is a necessary step in assessment (McCulloch, 2013).

The European Welfare Quality Project developed the Welfare Quality® framework to integrate animal welfare into the food quality chain (Canali and Keeling, 2009). The project created practical on-farm welfare assessment tools for pigs, poultry and cattle and reliable species-specific strategies to improve welfare (Canali and Keeling, 2009; Perny et al., 2009). The resulting framework is based on the Five Freedoms and identifies 12 key welfare criteria, classified under four main welfare principles: good feeding, good housing (sometimes modified to “good environment” to reflect the fact most sheep are rarely housed, for example), good health, and appropriate behaviour (AWIN, 2015; Perny et al., 2009). This work helped establish animal-based indicators as the gold standard for welfare assessments by showing that they provide direct information about the animal, and therefore an accurate representation of its welfare (AWIN, 2015; EFSA, 2012; Richmond et al., 2017; Winckler et al., 2003). This formed the basis for future research in on-farm welfare assessment methods, including the

European Animal Welfare Indicators Project (AWIN), which published welfare assessment protocols for sheep and goats, among other species (AWIN, 2015, 2015a).

The Five Domains Model was developed in 1994, partly to address the challenges faced by the Five Freedoms framework (Mellor & Reid, 1994). The framework was published to assess the impact of an experiment on an animal (Mellor & Reid, 1994). It described "domains of potential compromise" rather than an ideal welfare state (Mellor & Reid, 1994). By doing so, The Five Domains went beyond evaluating functional disruptions or behavioural restrictions and used negative affective states as a measure of overall experiment impact on welfare (Mellor, 2016). The model has been updated many times to reflect the evolution of animal welfare science (Mellor et al., 2020). In the model's first iteration published in 1994, the five domains were nutrition, environment, health, behaviour and mental state (Mellor et al., 2020). The principle was to use the objective evidence collected when assessing the first four domains to infer the subjective, affective experiences belonging to the fifth domain (Mellor et al., 2020). In 2015, the model's first four domains were revised to include factors leading to positive affects, based on the contemporary scientific evidence being published that examined animals' ability to have pleasurable experiences (Mellor et al., 2020). Finally, in 2020, the fourth domain was renamed "Behavioural Interactions" to reflect the agency-related behaviours animals use to respond to external stimuli, especially to humans (Mellor et al., 2020). The new model factors in human-animal interaction and the paper describing it provides examples of interactions likely to generate negative and positive affects (Mellor et al., 2020).

This thesis grounds itself in the Five Domains framework, because although my work centres around negative affective states brought on by disease, indicators of positive welfare were measured and questions about the fifth domain, affective experiences, were at the core of my hypotheses. The framework was used in a conceptual way to determine animal welfare indicators that were of interest to study. It also guided the discussion around the importance of addressing the conditions I studied. They have the potential to cause pain, discomfort and changes in behavioural expression, as will be presented throughout this thesis, and therefore have a negative impact on the affective experience domain (Mellor, 2017).

1.2 Measuring Welfare Challenges in Extensive Systems

Among the wide range of welfare challenges faced by sheep in extensive systems, this thesis will concentrate on lameness, gastrointestinal parasitism and mastitis. These conditions have important economic, health and welfare impacts. In the UK, sheep lameness costs the industry between £80 and £84 million annually (Wassink et al., 2010). At a flock level, it is estimated to cost between £3.90 and £6.35 per ewe per year (Green et al., 2012; Winter & Green, 2017). Economic losses due to helminth parasitism are estimated at £45 million every year (Charlier et al., 2020). Meanwhile, a 10% reduction in mastitis could save £2.7 million in the Texel breed alone (Conington et al., 2008). Beyond their significant cost, lameness and parasitism have a high prevalence and mastitis, though not as prevalent, can be very acute and painful (Best et al., 2020; Charlier et al., 2020; Conington et al., 2008). A farmer survey reported that lameness was present on 90% of UK farms with a mean prevalence of 3.2% of ewes being lame (Best et al., 2020). Gastrointestinal parasite infection is ubiquitous in grazing sheep, and treatment relies on regular anthelmintic treatments (Morgan & van Dijk, 2012). As resistance to common anthelmintic drugs increases, the risk of clinical disease rises (Barger, 1999). In a 2011 survey, lameness and parasitism were identified as priority welfare concerns by 53% and 74% of experts, respectively (Phythian et al., 2011). Although there is no general consensus on the prevalence of mastitis in ewes in the UK, it is known to be a painful condition and is considered a top welfare priority by veterinarians and animal welfare professionals (Fitzpatrick et al., 2006; Rioja-Lang et al., 2020a). Furthermore, a UK meeting held by the EU research project TechCare (which this thesis is funded by) found that stakeholders ranked lameness and parasitism as some of the most important welfare concerns faced by sheep (Dwyer et al., 2021).

In the past, welfare was often assessed using environmental or resource-based factors (Winckler et al., 2003). For example, the type of housing or bedding available would be evaluated. Most commercial welfare assessment schemes and regulations rely on these kinds of resource-based indicators to measure the inputs in the system where the animals are being raised (Smulders & Algers, 2009). This is because input-based measures are quick, easy, and reliable (Waran & Randle, 2017). However, the wide range of management systems and environments in which sheep are raised can render these indicators impractical (Richmond et al., 2017). Although inputs should be considered as risk factors that might affect welfare, there are many other factors that should not be overlooked (AWIN, 2015; Smulders & Algers, 2009). Animal-based, or outcome-based, measures (ABMs) are better suited to inform us on the resources' actual effect and indicate the animals' current welfare state (Smulders & Algers, 2009). The EFSA considers ABMs to be "the most appropriate indicators of animal welfare and a carefully selected combination of animal-based measures can be used to assess the welfare of a target population in a valid and robust way" (EFSA, 2012). Where ABMs are unavailable or unfeasible in a farm setting, resource-based measures can be presented as a valid option (Richmond et al., 2017). For example, assessing the absence of prolonged thirst can be done by examining water availability (AWIN, 2015; Richmond et al., 2017). Sheep welfare in extensive systems can reliably be assessed using ABMs, although some behavioural indicators require further reliability testing (Richmond et al., 2017).

Since behavioural symptoms are often visible before clinical signs of disease, non-invasive studies of sheep behaviour can provide direct insight into the animals' experiences of welfare challenges (Gougoulis et al., 2010). For example, changes in feeding and grazing behaviour are often signs of gastrointestinal abnormalities, while changes in posture, activity levels and locomotion are the most common sickness behaviours in ruminants (Borderas et al., 2008; Gougoulis et al., 2010). Sickness behaviours are evolved, adaptive responses to illness and are recognised as indicators of infection or stress and compromised welfare (Hart & Hart, 2019). Decreased exploration, social and sexual behaviours can be part of a coordinated response designed to help animals recover from illness (Weary et al., 2009). A reduction in social behaviour has been reported in sick farm animals, including dairy calves, dairy cows and sheep (Borderas et al., 2008; Morris et al., 2022; Weary et al., 2009). Behaviours that provide long-term fitness and welfare benefits, such as play or grooming, are most likely to decrease as resources are diverted to critical behaviours offering short-term value (Borderas et al., 2008; Hart & Hart, 2019; Weary et al., 2009). Because the ewe-lamb bond is so important for lamb survival, measuring it could provide researchers with important welfare information about the lamb and the ewe (Dwyer, 2014). Numerous behaviours are linked to the pain response and can be used as indicators of levels of pain being experienced by animals (Prunier et al., 2013). In mammals, behavioural indicators of pain include vocalisations, tonic immobility and aggressiveness (Prunier et al., 2013). Pain behaviours in lambs have been described as consisting of rolling, writhing, kicking, stamping, tail-wagging, lip-curling and licking or biting the site of damage (Molony & Kent, 1997). More passive pain behaviours take place after the prolonged active response tires the lamb; abnormal posture, refraining from movement and standing or lying completely still can indicate chronic pain (Molony & Kent, 1997). Because immobility avoids stimulating damaged tissue, extreme stillness is seen as a sign of very severe pain in lambs (Molony et al., 1993). However, substantial individual variation in the behaviours displayed can lead to inaccuracies in behavioural observations (Thornton & Waterman-Pearson, 1999).

Research on sheep and cattle suggests that the monitoring of the circadian rhythm of animals' activity could help detect disturbances caused by disease (Nunes Marsiglio Sarout et al., 2018; Plaza et al., 2022; Wagner et al., 2021). Patterns that shift between day and night and throughout the seasons have been identified in the timing, speed and direction of sheep's grazing activity (Nunes Marsiglio Sarout et al., 2018; Plaza et al., 2022). Disturbances in these patterns, for example those caused by changes in weather, have successfully been recorded by activity sensors worn by sheep (Nunes Marsiglio Sarout et al., 2018). In cattle, a mathematical method has been developed for an indoor positioning systems that can detect 95% of disease (acidosis, lameness, mastitis, and others) and reproductive events (oestrus, calving) that disturb the animals' circadian rhythm (Wagner et al., 2021). Changes in circadian patterns of behaviour would be difficult to monitor through in-person observation. Technology such as wearable activity sensors could help monitor animal behaviour patterns over time and provide insights into disease-related welfare problems.

Another type of behavioural assessment using qualitative methods was first described in a study observing the behaviour of pigs (Wemelsfelder et al., 2000). Qualitative Behaviour Assessment (QBA) records the expressive qualities of animals' general demeanor using terms such as “fearful”, “agitated”, or “calm” (Phythian et al., 2013). Thus, it can offer behavioural descriptions of affective states, such as fear, agitation or calmness (Wemelsfelder & Farish, 2004). Quantitative measurements are indirect and offer no certainty that measures always reflect the same affective state (Wemelsfelder & Farish, 2004). For example, the behaviour of cull ewes coming off the transport lorry has been described as “active and alert,” (Knowles, 1998) but this may not be a good indicator of their welfare because it is unclear whether this high level of activity was explorative and calm rather than agitated and fearful (Wemelsfelder & Farish, 2004). The behavioural judgements that result from QBA are used as accurate empirical information to study the emotional expression of sheep (Wemelsfelder & Farish, 2004). Studies have concluded that it is a valid and feasible methodology to assess sheep welfare in a variety of systems (Diaz-Lundahl et al., 2019; Phythian et al., 2013; Wickham et al., 2012). It is included in the Welfare Quality® Protocols and in the Animal Welfare Indicators Project's (AWIN) Welfare Assessment Protocol for Sheep (AWIN, 2015) and has become a valuable tool for animal welfare scientists (Cooper & Wemelsfelder, 2020; Keeling et al., 2013). It is one of the few welfare assessment techniques that can measure positive animal welfare (Cooper & Wemelsfelder, 2020; Fleming et al., 2016).

1.3 Lameness, Gastrointestinal Parasitism, and Mastitis as Welfare Concerns

During the early stages of an immune response, the mammalian immune system produces pro-inflammatory cytokines (Nordgreen et al., 2020). These cause behavioural changes such as anorexia, lethargy and decreased social motivation (Hart, 1988). Measuring the behavioural changes that occur during the sickness response may be a way of identifying welfare concerns before clinical signs of disease are obvious and the animal's welfare is further compromised.

1.3.1 Lameness

While lameness is the manifestation of a range of conditions, footrot and interdigital dermatitis account for 80% of sheep lameness in the UK (Kaler & Green, 2008; Nalon & Stevenson, 2019). Lameness can cause low body weight, reduced growth rate and reduced wool growth (Marshall et al., 1991). Production and longevity are reduced in lame sheep as they are more likely to die or be culled prematurely (Nalon & Stevenson, 2019). Lame sheep experience pain, violating one of the core tenets of all welfare assessment frameworks (Fitzpatrick et al., 2006; Ley et al., 1989; Winter, 2008). Lame sheep can experience hyperalgesia, an indirect indicator of pain (Ley et al., 1989). It has been suggested that lameness prevalence can predict the likelihood of ewes having their future welfare compromised (Munoz et al., 2018). Early detection and treatment of lameness are key to reducing its prevalence (Kaler et al., 2020), meaning that early indicators of lameness are crucial to improve sheep welfare in the long term.

Validated lameness scoring systems and their relevant training programs could encourage producers to prevent and treat lameness promptly (Nalon & Stevenson, 2019). However, this type of data is difficult to collect from extensive flocks at regular intervals, rendering it an impractical indicator for lameness detection (Kaler et al., 2020; Munoz et al., 2018). Studies in cattle have found that lame cows spending more time lying in fewer, longer bouts, tend to have lower body condition scores (BCS) and reduce their pedometric activity by at least 15% (Barwick et al., 2018; Green et al., 2014; Westin et al., 2016). Changes in walking behaviour and gait can sometimes be observed in lame sheep (Kaler et al., 2020). However, the trend towards reduced numbers of stockpeople on farms makes visual observation difficult (Barwick et al., 2018). Additionally, because sheep are prey animals, they often hide signs of lameness in the presence of humans (Kaler et al., 2020). In extensive systems, the visual observation of subtle or even evident behavioural changes is impractical and sometimes impossible (Kaler et al., 2020).

In 2011, FAWC set a target of less than 2% sheep lameness across UK farms by 2021 (FAWC, 2011). Progress has been made through promotion of best practices to farmers (Best et al., 2020); lameness prevalence in UK sheep flocks was halved between 2004 and 2013, from 10.6% to 4.9% (Kaler & Green, 2009; Winter et al., 2015). But the gold-standard of visual observation for lameness detection remains subjective and time-consuming (Busin et al., 2019). Measuring indicators of lameness through technology could be key to the further reduction of this condition to meet and surpass the established targets. Such use of technology is much more common in other species, such as dairy cattle, than in sheep. A systematic review reported on three commercially available methods of cattle lameness detection: pressure plates/load cells, image processing techniques and activity-based techniques (Silva et al., 2021). Each of these methods were supported by many published studies (Silva et al., 2021). There are no commercially available products for sheep lameness detection and, although research in this field is growing, the body of research remains comparatively small compared to cattle (Spigarelli et al., 2020). A study linking environmental factors such as bedrock class and selenium levels in soil to lameness using a smartphone app to record lameness levels was able to draw conclusions about the epidemiology of lameness-causing diseases (Vittis & Kaler, 2020). Research teams have successfully used tri-axial accelerometers and machine learning techniques to differentiate lame sheep from sound ones based on the length, variability and smoothness of activities such as walking, standing and lying (Barwick et al., 2018; Kaler et al., 2020). However, the devices were attached to sheep in ways that would not be feasible in a commercial production system (i.e. tape around ear tags and veterinary bandaging around legs). Micro-Doppler radar paired with machine learning algorithms was used to detect lameness to 96% sensitivity and 94% specificity, although only eight sheep were tested (Busin et al., 2019). These studies served as important proof-of-concept trials but their methods do not provide practical solutions for commercially-viable products.

1.3.2 Gastrointestinal Parasitism

Endoparasitism is most often clinically identified using faecal egg counts (FEC) or adult worm counts during necropsies (Asmare et al., 2016; Karrow et al., 2014). DISCO and FAMACHA scores which measure faecal consistency and eyelid membrane colour respectively, were created to identify sheep likely to be infected with helminths (Bentounsi et al., 2012; Cabaret et al., 2006; Van Wyk & Bath, 2002). Liveweight gain is reduced in infected animals and carcasses are of lower quality at slaughter (Coop et al., 1977, 1988; Coop et al., 1982; Hubert et al., 1979). There are many studies describing the immunological and metabolic effects of endoparasites. Infection leads to protein deficiency through a reduced supply of amino acids due to appetite suppression combined with an increased demand for protein in the alimentary tract (Sykes & Coop, 2001). Many immune system components, such as immunoglobins, mast cells and globule leukocytes rely on metabolised protein resources (Coop & Holmes, 1996). This negative energy balance can lead to a reduced local immune response, making ewes more vulnerable to other infections such as mastitis (Kordalis et al., 2019). Infection with *Teladorsagia circumcincta,* the most common parasitic nematode in temperate areas like the UK, leads to damage in the abomasal mucosa, which is defined as inner wall lesions in the ruminant abomasum, and an increase in pepsinogen concentration in the plasma (Coop et al., 1985; McKellar, 1993). Pepsinogen concentrations could therefore be a useful tool in recognising abomasal damage brought on by infection. However, there are limitations to its use as an indicator of infection: differences in worm length can lead to variation in pepsinogen concentration, it can also be a sign of gastric ulcers rather than parasite infection and increased pepsinogen can be recorded despite low levels of larvae establishment in older immune ewes (Stear et al., 1999, 2003; Yakoob et al., 1983). The potential value of pepsinogen levels as a welfare indicator has yet to be investigated.

Potential behavioural effects of infection in sheep include parasite-induced anorexia and changes in diet selection (Hutchings et al., 1999; Jones et al., 2006). Where *Teladorsagia* are the dominant parasites, measures of appetite could act as indicators of infection-induced inappetence as one of the initial clinical symptoms (Kenyon & Jackson, 2012). Anorexia is both a result and a response to parasitism (Kyriazakis, 2014). The reduction in feed intake is due to the animal's immune response as well as the abomasal damage brought on by gastrointestinal parasites (Coop et al., 1985; Greer et al., 2008; Szyszka et al., 2013). The extent of anorexia is thought to increase with greater larval challenges (Laurenson et al., 2011). Studies have found that voluntary feed intake began to decline around the fourth week following infection with *Trichostrongylus colubriformis* in lambs (Kyriazakis et al., 1996). At the same time, moderate numbers of eggs were detectable in the faeces (Kyriazakis et al., 1996). This suggests that a minimum of established adult worms is needed for inappetence to develop (Kyriazakis et al., 1996). Parasitised sheep had a lower daily herbage intake due to shorter grazing bouts than non-parasitised sheep (Hutchings et al., 2000). A reduction in activity levels has been reported in parasitized sheep, which was moderated when they were mixed with non-parasitised sheep on pasture (Morris et al., 2022). In a wild population of Soay sheep, *T. circumcincta* infection contributed to a major mortality event in 1989 when it was combined with a widespread food shortage (Gulland, 1992). In cattle, posture and activity were affected to a variable extent by subclinical parasitism with *Ostertagia ostertagi* (Szyszka et al., 2013).

The lack of early indicators of parasitism in sheep was highlighted as early as 1982 (Coop et al., 1982). Since then, this knowledge gap has been addressed by the studies described above, but the effects of gastrointestinal parasitism on welfare have yet to be thoroughly explored. The levels of pain or discomfort experienced by sheep during subclinical infections remains unclear. Research suggests that any negative difference between the estimated and actual energy utilisation could be interpreted as affecting the animal's well-being (Greer et al., 2009). According to the Good Health tenet of the Welfare Quality framework, a parasitised sheep's welfare is compromised as it does not fit the "Absence of disease" welfare criteria (Perny et al., 2009). It is unclear whether other welfare criteria are met in parasitised sheep, such as "Comfort around resting," "Expression of social behaviours," "Expression of other behaviours," and "Positive emotional state" (Perny et al., 2009). In the Five Domains framework, it is unclear what kind of impact parasitism has on the Affective Experience Domain of sheep. Endoparasitism can cause varying degrees of abomasal damage (Coop et al., 1985; Szyszka et al., 2013). In veal calves, it is not known whether abomasal lesions cause pain as they are rarely accompanied by clinical signs, except in the most severe of cases where the abomasal wall is haemorrhaging or perforated (Bus et al., 2019). Some studies have found that abomasal ulcers cause pain in cattle, but parasites usually lead to nodules rather than ulcers and it is unclear if these are comparable (Bus et al., 2019; Kuiper, 1991; Munch et al., 2019). In summary, the literature is inconclusive about the experience and affective states of parasitised ruminants. It is agreed that it leads to reduced feed intake and an immune response, but it is unclear if this leads to hunger or discomfort (Greer et al., 2008). It is suggested that abomasal damage may cause pain, but studies focus on production factors such as growth and fertility (Bus et al., 2019; Coop et al., 1988; Fthenakis & Papadopoulos, 2018). One study applying QBA on parasitised and non-parasitised sheep reported that observers scored the treatment groups differently, and concluded that QBA provided insight into the behavioural expression of parasitised sheep (Grant et al., 2020). As resistance to common anthelmintics becomes more widespread, the potential welfare implications of infection grow (Bartley et al., 2003; Jackson & Coop, 2000). Understanding the welfare costs of endoparasitic infection through behavioural indicators could help value it as a welfare issue as well as a production issue.

Further research on the welfare implications of parasitism is crucial, as several studies found that many stakeholders already consider it a welfare challenge, despite the lack of explicit evidence in the welfare literature. In a study using the Delphi method to ask experts to rank welfare concerns for various species, gastrointestinal parasitism was ranked 4^{th} and 5^{th} for severity and prevalence, respectively (Rioja-Lang et al., 2020a). In a stakeholder meeting for the TechCare project, parasitism was ranked as the most important welfare concern faced by sheep (Dwyer et al., 2021). A similar TechCare meeting held with Romanian dairy sheep farmers also listed gastrointestinal parasitism as one of their top welfare concerns (Czizster et al., 2022).

1.3.3 Mastitis

Most published literature on mastitis refers to dairy breeds of cattle and sheep, and there is little information on meat sheep breeds. Nevertheless, mammary infections are an important welfare concern in meat production flocks (McLennan et al., 2016). The pathogens responsible for mastitis can cause painful lesions in the teat canals and disease can develop very quickly (Mavrogianni et al., 2004; McLennan et al., 2016). Incidence of clinical mastitis is associated with increased ewe and lamb mortality in meat breeds (Arsenault et al., 2008; McLennan et al., 2016) and mastitic ewes have been reported to suffer from mechanical hyperalgesia, indicating a clear welfare issue (Dolan et al., 2000). Reduced milk yield of infected ewes leads to suboptimal growth of their lambs (Gelasakis et al., 2015) and potential subsequent removal of the ewe from the breeding flock (Conington et al., 2008). Severe clinical cases of mastitis cause high levels of pain in dairy cattle and algorithms using facial pain scales reliably identified sheep with mastitis, suggesting that the condition is also perceived to be painful by ewes (Leslie & Petersson-Wolfe, 2012; McLennan et al., 2016).

While clinical mastitis is a widely acknowledged welfare issue in sheep flocks in the UK (EFSA, 2014; Rioja-Lang et al., 2020a), the effect of subclinical mastitis on sheep welfare is less clear. It is more prevalent than its clinical form in most dairy animal populations, representing up to 95% of mastitis cases in sheep (Martins et al., 2013; Sinha et al., 2018). Diagnosis relies on milk-based measures which are difficult to obtain in meat breeds such as somatic cell count (SCS) or the California Mastitis Test (CMT) (Conington et al., 2008; Sinha et al., 2018). There is a need to identify practical indicators of subclinical mastitis and to determine its impact on meat sheep welfare in extensive systems.

When assessing sheep welfare, it is as important to consider the risks of future welfare compromise as the actual welfare concerns (Richmond et al., 2017). This is especially true in extensive systems where monitoring is infrequent (Richmond et al., 2017). Since subclinical cases of mastitis sometimes lead to clinical cases, they can be seen as equally important welfare concerns (Arsenault et al., 2008; Watkins et al., 1991). Practical animal-based indicators of subclinical mastitis remain sparse. While environmental factors such as temperature and precipitation have been linked to the risk of subclinical mastitis cases in dairy sheep, these measures are too broad to systematically identify cases (Giannakopoulos et al., 2019). Studies have identified risk factors that increase the odds of subclinical mastitis in meat ewes, such as having three or more lambs, and ewes being multiparous (Arsenault et al., 2008; Torres-Hernandez & Hohenboken, 1980; Watkins et al., 1991). Research in dairy breeds suggests that a reduction in lamb sucking behaviour can act as a behavioural indicator of subclinical mastitis in ewes (Gougoulis et al. 2008) while technology such as infrared images obtained by thermographs can diagnose subclinical mastitis based on udder temperature, although this technique requires handling and specific expertise (Gougoulis et al., 2008; Martins et al., 2013). Future research identifying practical early indicators of mastitis could contribute to the improvement of farm productivity and animal welfare.

1.4 Precision Livestock Farming (PLF)

1.4.1 The Potential of PLF

This thesis will examine the role technology can play in the early detection of welfare concerns alongside more traditional observation methods. Behavioural changes are often the first signs of disease, but human observation is time consuming, labour intensive and can alter animal behaviour (Gougoulis et al., 2010; Healy et al., 2002; Kaler et al., 2020; McLennan et al., 2015; Szyszka et al., 2013). Effective monitoring is becoming more difficult as the skilled labour supply on farms dwindles and the sheep to stockperson ratio increases (Goddard et al., 2006; Richmond et al., 2017). This leads to welfare concerns not being addressed promptly, if at all (Richmond et al., 2017). Neglect and lack of stockpersonship with good knowledge and skills were identified by experts as priority welfare issues for farm animal species (Rioja-Lang et al., 2020).

This creates an opportunity for the application of Precision Livestock Farming (PLF). It is defined as managing individual animals by continuous real-time monitoring of health, welfare, production, reproduction, and environmental impact, essentially every aspect of a farmed animal's life (Berckmans, 2017). No living organism truly acts as the theoretical average of a group. On the contrary, they have a "complex, individually different, time-varying and dynamic" (CITD) nature (Berckmans, 2017). Algorithms for machine learning should be developed based on this CITD nature, not on theoretical averages used to compare groups in statistical analyses (Berckmans, 2017).

However, it is currently unrealistic to expect every ewe and lamb in a flock to be fitted with individual, wearable sensors to monitor their individual welfare. Indeed, it may be difficult to envisage any PLF tool being developed and sold at a cost that would lead to an attractive return on investment for current UK sheep farmers, seeing as they have the lowest profit margins of all livestock enterprises (DEFRA, 2014; Kaler & Ruston, 2019). Wathes et al. (2008) defines PLF as the management of livestock production using the principles and technology of process engineering. This thesis recognises that rather than strictly recording data at the individual level, PLF can be applied at the pen, barn, or flock level (Wathes et al., 2008). This is due to the high cost of technology that often renders impossible applications at the individual level, except in animals of high value, such as sows or dairy cows (Wathes et al., 2008). It is further described as considering livestock farming as a series of linked processes within a complex system (Wathes et al., 2008). Notably, animal behaviour is identified as one such process suitable to the PLF approach (Wathes et al., 2008).

A review of welfare measures for ruminants reported that studies using sensors to monitor health and welfare have seen a marked increase in publication numbers since 2015 (Spigarelli et al., 2020). The authors of this review hypothesise that this growing number of studies assessing welfare with sensors is partly due to the increased interest of welfare scientists and consumers in pasture-based systems (Spigarelli et al., 2020). Although devices such as accelerometers for lameness detection are commercially available for dairy cows, there is a lack of technology in use by sheep farmers (Barwick et al., 2018). Recent research into PLF applications in sheep often resort to custom made pieces of equipment or adapting tools created for other species (Barwick et al., 2018; Högberg et al., 2020; Kaler et al., 2020; Mason & Sneddon, 2013). This can result in large, unwieldy datasets and injuries can arise from putting tools for other species on sheep. Devices for assessing welfare are often designed with intensive management systems in mind, whereas most sheep in the UK are kept in extensive or semi-extensive conditions (EFSA, 2014; Turner & Dwyer, 2007). Technology is generally seen by UK sheep producers as costly, difficult to use, and offering few benefits (Kaler & Ruston, 2019; Lima et al., 2018). Many British producers feel pressured by the government to adopt new tools such as EID (Electronic Identification) for management purposes, in turn strengthening their negative perception of PLF (Lima et al., 2018).

1.4.2 PLF successes

Despite lagging behind other species, the application of PLF techniques to monitor sheep health has started and studies suggest that the use of technology could have a tangible, positive effect on profitability (Lima et al., 2018; Morgan-Davies et al., 2018). For example, in a trial on a Scottish hill farm, existing technologies such as EID and a 5-way Auto Draft weigh crate paired with an algorithm were used to detect parasitised lambs (Morgan-Davies et al., 2018). They found they could reduce the proportion of lambs requiring anthelmintic treatment by 40%, leading to a drop of 46% in the total amount of anthelmintics used and an increase of up to £3 in net margins per ewe (Morgan-Davies et al., 2018). These results give an example of the value that PLF can bring to farm productivity and animal health, through reducing the risk of anthelmintic resistance (Morgan-Davies et al., 2018). Bluetooth technology has been successfully applied to determine maternal pedigree of lambs based on the mean distance between ewes and lambs (Sohi et al., 2017). If these PLF tools are developed and marketed in a way that appeals to sheep farmers, there is potential for it to have a large impact on the industry.

There have been some advances in identifying potential welfare issues using PLF. Tri-axial accelerometers and machine learning algorithms have successfully identified changes in behavioural patterns on the day of parturition in ewes (Fogarty et al., 2020). They have differentiated between lame and sound sheep (Barwick et al., 2018; Kaler et al., 2020) and between sheep parasitised with strongyles and non-parasitised sheep (Burgunder et al., 2018; Morris et al., 2022). The differences measured by these technologies to class sheep as healthy or diseased were often found to be undetectable through simple observation methods (Burgunder et al., 2018; Kaler et al., 2020). Past research has used algorithms to find differences in the range of long-term behavioural complexity of parasitised sheep compared to healthy ones (Burgunder et al., 2018). Other algorithms have identified differences in the variability and smoothness of ewes' lying, standing and walking movements (Kaler et al., 2020). Recent studies have found that not only did parasitism reduce sheep's activity levels but socialising with non-parasitised sheep moderated this effect on activity (Morris et al., 2022).

Some validation work has been conducted on the different types of accelerometers that are commercially available. The IceQube and IceTag (IceRobotics, Peacock

Technology Ltd., Stirling, UK) have been validated for recording lying and standing time, and activity levels in lambs (Högberg et al., 2020; Morris et al., 2022). The ActiWatch Mini (CamNtech, Cambridgeshire, UK) was validated for the detection of low and high activity in ewes (McLennan et al., 2015). This type of technology could contribute to early detection of disease, particularly in the absence of overt behavioural symptoms, which is often the case given that sheep are a prey species (Burgunder et al., 2018; Kaler et al., 2020). This could lead to earlier targeted treatments, better welfare and significant labour and cost savings (Barwick et al., 2018; Kaler et al., 2020; Morgan-Davies et al., 2018). However, validation of all technological tools must continue and remains a priority. Reports indicate that only 14% of commercial sensors for dairy cattle have external validation studies available and 23% of welfare-monitoring technologies for pigs are acceptably validated (Larsen et al., 2021; Stygar et al., 2021). The lack of validation of PLF tools was raised as a concern at farmer workshops (Schillings et al., 2021b). The commercial reality of needing to protect product and company information likely plays a role in the shortage of published independent validation studies. However, an increase in the percentage of validated technologies is required if PLF is to become a trustworthy tool for farmers and researchers.

1.4.3 PLF Technology Challenges and Opportunities

The use of accelerometers in livestock studies and on commercial farms is not without its challenges. Motion indexes return information about the total amount of movement performed by an animal, but the purpose of said movement remains unknown. Only with in-person observation or measurement of other variables can conclusions be drawn about the nature of the animal’s activity (Morris et al., 2022). For example, a reduction in motion index in sheep parasitised with a gastrointestinal parasites has been reported (Morris et al., 2022). Since anorexia and reduced feed intake are associated with the disease, the reduction in motion index may represent a reduction in grazing behaviour. However, this cannot be verified without focal observations of the sheep or a measure of forage intake (Morris, 2022). Another challenge for accelerometer users is the large amount of data being collected continuously while the tools are in use. Validated algorithms and user-friendly dashboards must be created for these data to be interpreted and applied on commercial farms (Schillings et al., 2021a). These large data sets collected over short periods of time combined with poor battery life make collection and storage of long-term information difficult.

PLF sensors have been used to measure proximity and social interaction in a range of species. They have been applied to measure social interaction in response to social stress in beef cattle (Patison et al., 2017). They have been used to study the social structure of a flock of ewes, showing that it is fluid, yet affected by environmental factors such as sudden weather changes (Ozella et al., 2020). Since changes in social motivation are a key feature of sickness behaviour, monitoring social interactions has the potential to detect disease (Proudfoot et al., 2012). The use of Bluetooth beacons in research and their implementation on farm is feasible thanks to their low cost and low energy use, leading to long battery life (Hasan et al., 2022). They can also transmit information live over wireless networks, meaning there is no need for the time-consuming task of removing the devices from the animals and downloading large datasets. One of the challenges research teams face when using Bluetooth beacons is that they cannot detect the context of a social interaction (Neethirajan & Kemp, 2021). It is impossible to deduce the valence of an interaction strictly through proximity data. Combining Bluetooth beacons with other types of technology, such as accelerometers or video recording, can provide some context for the interaction (Lee et al., 2016; Ozella et al., 2020). Proximity sensors can even be used to assess human-animal interactions, which are a key part of an animal's ongoing welfare (Neethirajan & Kemp, 2021).

Technological developments so far have mostly focused on health and production measures (Buller et al., 2020). The opportunity to place welfare at the forefront of innovative development of PLF tools should be seized (Buller et al., 2020). As technology evolves, so will the role of farmers and stockpeople (Buller et al., 2020). Daily contact with animals risks being reduced to reading data points on a screen (Buller et al., 2020). This could reduce instances of physical contact between humans and animals to unpleasant procedures and treatments, thereby increasing the negative response to human interactions (Buller et al., 2020; Cornou, 2009). On the other hand, if farmers' workload is lightened, it might lead to them spending more time close to their animals, tending to their welfare (Buller et al., 2020; Hostiou et al., 2017). Studies have found that regular positive contact with humans can reduce the stress response to routine husbandry procedures (Hayes et al., 2021). These types of findings suggest that physical contact with stockpeople is crucial to farm animals' welfare, and it should therefore not be reduced to the bare minimum.

Consumer perception of PLF will depend heavily on the industry's ability to prove its concrete, positive impact on animal and farmer welfare (Cornou, 2009). Specifically, it will become key to show that implementation of PLF does not lead to further objectification of animals (Cornou, 2009). One systematic review found that more publications on poultry PLF stated their study aims to be increased animal health and welfare rather than increased production (Rowe et al., 2019). Authors have commented on the fact that while PLF is currently able to monitor specific facets of animal welfare, such as the absence of hunger or lameness, the tools cannot yet provide a broad, multidimensional view of the animals' complete welfare state (van Erp-van der & Rutter, 2020).

Early in the development of PLF, researchers called for ethical studies into its potential uses (Wathes et al., 2008). When adding any device on an animal, there is a risk of injury to the animal. A bioethical study of automatic milking systems (AMS) for dairy cows is a pertinent example of the kind of analysis required as more technologies are rolled out (Millar & Mempham, 2001). In it, improvements to production efficiency and human and cow welfare are identified, but concerns over the instrumental use of animals are raised (Millar & Mempham, 2001). Providing a sound ethical basis for PLF would help ensure a greater chance of success for its applications (Wathes et al., 2008). If the roll-out of tools is rushed and results in unaffordable systems, inappropriate process models, or unreliable first products evaluated at the expense of the animals' welfare and farmers' pocketbooks, they will be rejected (Wathes et al., 2008). Additionally, the risks of inappropriate design should be considered, e.g. injury to the animal. As the future users of PLF, farmers should always form a central part of the development process through consultations and pilot trials to ensure their needs are being met by the product. Further research is required to understand the exact welfare benefits and pitfalls of PLF, as the current body of work is not sufficient to conduct a bioethical analysis (Wathes et al., 2008). Cooperation between interdisciplinary researchers should be encouraged to conduct this kind of work (Buller et al., 2020).

Though PLF is often positioned as a cost saving tool to producers, it has great potential to improve welfare management, especially in extensive systems where monitoring individuals is difficult (Fuchs et al., 2019; Munoz et al., 2018; Richmond et al., 2017). No commercially available technology can currently identify individual sick sheep in an outdoor environment. Therein lies an opportunity to describe novel or known animal-based indicators for the main welfare concerns faced by sheep that could be read by PLF technology. Once these indicators are identified, a reliable and practical PLF system needs to be developed.

1.5 Aims of this thesis

This thesis project was part of the TechCare project, funded by EU Horizon 2020. TechCare is a multi-actor project aiming to develop business models using innovative and precision technologies to improve welfare management in sheep and goats in Europe. The project team consists of 19 partner institutions from eight countries, working on eight different work packages. The primary research questions of this thesis were: Which welfare indicators could be measured by PLF technology to monitor and manage sheep welfare? And which PLF technologies have the potential to measure these indicators?

More specifically, the aims of this thesis were to:

- Determine the welfare impact of gastrointestinal parasitism on lambs and pilot the use of behavioural indicators of welfare in a controlled environment (Chapter 2).
- Identify behavioural indicators of lameness, gastrointestinal parasitism, and mastitis in semi-extensively managed sheep, since these are three of the main welfare concerns on commercial farms, through in-person observations (Chapter 3).
- Validate the use of accelerometers to differentiate across sheep behaviours for the purpose of measuring behavioural indicators of welfare and determine whether wearing collars containing technology impacts the behaviour and welfare of sheep (Chapter 4).
- Test the use of Bluetooth beacons to identify patterns in ewe-lamb distance associated with poor welfare (Chapter 5).
- Identify the motivations of farmers using PLF tools, their preferences and the barriers to its uptake (Chapter 6).

Chapter 2. The impact of parasitism on the behaviour and welfare of weaned housed lambs

Chapter 2 is the manuscript of a paper submitted to Applied Animal Behaviour Science on November 17th, 2023. It is currently under review. This chapter is therefore presented in the style of Applied Animal Behaviour Science. My contributions to this paper include behavioural sampling methods, data collection, data analysis and writing. Co-authors contributed experimental design, data collection and review and editing of writing.

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Abstract

Gastrointestinal parasitism is an important health and production concern in sheep, yet its impact on animal welfare is unclear and its effect on behaviour has only briefly been described. The impact of subclinical infections is especially ambiguous as parasitism often remains undiagnosed until clinical signs such as diarrhoea are evident. This study applied both quantitative and qualitative methods to examine the effects of subclinical *Teladorsagia circumcincta* infection on the behaviour and welfare of 96 Suffolk-cross lambs (24 pens of 4 lambs) divided into three treatment groups at the pen level: ad-lib fed control (AC), restricted-fed control (RC), and ad-lib fed parasitised (AP). Parasitised lambs were trickle dosed three times weekly with 7000 third stage larvae (L_3). Lambs in the RC group were pair fed to match AP feed intake to separate the effects of infection-induced anorexia from the potential direct impacts of infection. From 7 days pre-infection to 23 days post-infection, scan samples and focal observations were taken from video recordings to monitor lying, standing, eating, play and social behaviour, while animal-based measures such as faecal soiling score (FSS) were recorded as welfare indicators. Qualitative behaviour assessment (QBA) was conducted weekly to gain insight into the lambs' affective states over the onset of infection. The probability of AP lambs standing was greater than that of AC lambs over time ($p=0.006$). The probability of eating behaviour during the third daily scan sample was lower in AP lambs than in RC lambs ($p<0.001$). The FSS of all treatment groups increased over day of infection, but there was no significant difference across treatment groups. Principal Component Analysis (PCA) of the QBA data revealed that PC1 described arousal levels, from `Calm` to `Active`. I interpreted that PC2 described the valence of the animals' affective states, from `Agitated` to `Content` and PC3 described fearfulness and aggression levels, from `Aggressive` to `Alert`. Treatment group had no significant impact on the distribution of treatments on PC1 or PC2. However, AP lambs (est=10.64,SE=0.33) scored higher than RC lambs (est=9.42, SE=0.33) on PC3, the fearfulness dimension ($p=0.030$). There were no differences between fearfulness scores of AC and AP lambs or RC lambs. These results suggest that the early stages of subclinical *T. circumcincta* parasitism could have an impact on lamb standing and feeding behaviour, which have potential to be used as early indicators of disease. Infection may have increased the fear and anxiety levels of the lambs, thus impacting their welfare.

Keywords

Sheep, parasitism, animal welfare, behaviour, gastrointestinal, QBA

2.1 Introduction

Gastrointestinal (GI) parasitism is a known health and production concern in ruminants that is ubiquitous when animals graze on pasture (Charlier et al., 2020). However, its effect on behaviour can be difficult to monitor in extensive management systems and its impact on sheep welfare is unclear. If lambs adjust their behaviour in the early stages of infection before clinical signs are visible, it may be possible to use these behavioural adjustments as early indicators of parasitism. Identifying these early indicators could therefore lead to prompt treatment and improve overall health through reducing severity of infection. Aiming to identify potential early indicators increases the interest in subclinical infection and the early stages of parasitism. Understanding the welfare costs of GI infection could help evaluate its impacts on sheep mental state, as well as production. The levels of discomfort experienced by sheep during subclinical infections remains especially unclear as parasitism is often not diagnosed until clinical signs, such as diarrhoea, are evident. There is some evidence of altered affective state in sheep when infected with Strongylids, as reported in a study that found that observers performing qualitative behaviour assessment (QBA) described parasitised sheep as more "depressed/suspicious" before anthelmintic treatment and more "unsettled/apprehensive" than non-parasitised sheep (Grant et al., 2020). A 2020 study found that UK experts consistently ranked parasitism highly in a list of welfare concerns faced by sheep (Rioja-Lang et al., 2020a). The experts were especially concerned by the high prevalence of parasitism in the UK (Rioja-Lang et al., 2020a). This sentiment was echoed in a meeting of stakeholders involved in a large European project (TechCare, European Union's Horizon 2020 (No. 862050)), which included eight farmers out of the eleven participants, where GI parasitism was ranked as the second most important welfare concern for sheep in the UK (Dwyer et al., 2021).

Reliable tools to measure the effect of parasitism on welfare are needed to manage it effectively. Infection is ubiquitous in grazing sheep, and treatment relies on regular anthelmintic treatments (Morgan & van Dijk, 2012). As resistance to common anthelmintic drugs increases, the risk of clinical disease rises (Barger, 1999). By identifying and treating only infected sheep, in refugia populations of parasites are preserved and the efficacy of anthelmintics is prolonged (Kenyon et al., 2009). Therefore, early identification tools for individual infected sheep have become important to avoid blanket treatments of entire flocks. Additionally, considering the affective state of sheep during GI parasitism may contribute to our understanding of sickness behaviour pathways. For example, understanding when and why sheep experience discomfort could contribute to explaining their behavioural or dietary changes. To do so, the present study grounds itself in the Five Domains welfare framework, which uses affective states as a measure of the experiment's overall impact on welfare (Mellor, 2016). The five domains are nutrition, environment, health, behaviour and mental state, and it is the dynamic interaction between all these domains which can provide a systematic assessment of animal welfare at a given time (Mellor et al., 2020).

Animal-based indicators are the most appropriate tools to provide insight into the current welfare state of animals (EFSA, 2012; Smulders & Algers, 2009). Since behavioural symptoms are often visible before clinical signs, non-invasive studies of sheep behaviour can provide some direct insight into the animals' experiences of welfare challenges (Gougoulis et al., 2010). Sickness behaviours such as depression and anorexia act as a trade-off with social, feeding and mating behaviours to encourage recovery (Hart, 1988). For example, changes in posture, activity levels and locomotion are the most common sickness behaviours (Borderas et al., 2008; Gougoulis et al., 2010) and could act as potential welfare indicators. Methods such as QBA allow us to complement these approaches by directly assessing animals' mental states. Since GI parasites are globally ubiquitous, there is a need to understand this impact if we are to manage and improve sheep welfare. By collecting data on the health, behaviour and mental domains of parasitised lambs and consulting past studies on the effects of parasitism on the nutrition and environment domains, we aim to gather information on lambs' mental states during the early stages of infection.

The lack of early indicators of parasitism in sheep was highlighted as early as 1982 (Coop et al., 1982). Since then, studies have found that potential behavioural effects of GI parasitism include anorexia, and changes in diet selection, grazing and social behaviour. In one study, subclinically parasitised sheep reduced their bite rates and grazing depths, leading to a reduced risk of further infection (Hutchings et al., 1999).

This reflects findings based on behavioural observations of Soay sheep, which reported that heavily parasitised ewes may avoid grazing areas where the risk of parasitism is high (Hutchings et al., 2002). In an experiment observing grazing behaviour in sheep infected with *Teladorsagia circumcincta*, parasitised sheep spent less time grazing each day and had a lower feed intake than non-parasitised sheep due to their shorter grazing bouts (Hutchings et al., 2000). A study modelling sheep behaviour over the course of GI nematode infection highlighted the importance of grazing behaviour as a key factor in accurate GI parasite models (Fox et al., 2013). Targeted selective treatment is a method of identifying and treating individual infected animals based on easy to measure production factors such as milk production or live weight gain, which has been found to reduce anthelmintic use in different farm environments (Kenyon et al., 2009). Most recently, Morris *et al. (*2022) found that parasitised lambs had lower activity levels and fewer social interactions than non-parasitised lambs.

The aims of this study were to determine the effects of subclinical nematode infection on lamb behaviour to identify early indicators of GI parasitism, and to explore its impact on lamb welfare. We hypothesised that infected lambs would reduce their activity levels, feeding behaviour and social behaviour compared to non-infected lambs. They would have higher faecal soiling scores (FSS) and lower gut fill scores than non-infected lambs. Finally, we hypothesised that QBA would capture infected lambs' negative affective state, through higher scores on terms like `listless` and `apathetic,` for example.

2.2 Methods

2.2.1 Ethical approval

Ethical approval for this study was granted by SRUC's Animal Experiment Committee, as a subset of a larger experimental trial (AE Number: SHE AE 03-2021). All work is reported to be fully compliant with the ARRIVE2.0 guidance.

2.2.2 Animals

Ninety-six Suffolk cross male (48) and female (48) lambs were studied in this experiment. Eighty-four were Suffolk X Texel and the remaining twelve were Suffolk X

Blueface Leicester lambs balanced across the three treatment groups described below. All but five of the lambs were twins so the singletons were balanced across treatment groups. They were born on the experimental farm and stayed with their dams until weaning at 10 weeks of age. All lambs had tails docked and males were castrated. They were housed indoors until the experiment began when they were 4 months of age to ensure they were naïve to GI parasites. Prior to the start of the study, they were fed commercial pelleted feed (Tarff Valley Ltd., Castle Douglas, UK). During the study, their diet was made up of grass pellets (For Farmers UK Ltd., Bury St Edmunds, UK) consisting of 939 g/kg of dry matter and 122 g/kg DM of crude protein. They were housed in a shed where 24 pens were made of metal railing in blocks of four, each block being separated by a walkway. Lambs were kept in groups of four according to their treatment in the pens that measured $10m^2$, meaning each lamb had a space allowance of $2.5m^2$ per lamb. Each pen contained at least four feeders and one drinker, with saw dust bedding. Once restrictions were in place, RC pens had 5 feeders to minimise fighting. Two weeks into the study, one brush head was mounted on the inside of the gates of each pen, but these were removed after a week due to the lambs potentially ingesting brush bristles.

2.2.3 Experimental Design

There were three experimental treatments with 8 replicates, each consisting of a pen of 4 lambs balanced for breed and live weight. The treatment groups were ad-libitum fed control (AC), restricted-fed control (RC), and ad-libitum fed parasitised (AP). The latter were trickle dosed three times per week with approximately 7000 *T. circumcincta* L_3, a dose known to lead to subclinical infection (Coop et al., 1982; Fox et al., 2018). The AC and RC groups were sham infected with 4 ml of water, following the same protocol as the AP group. *T. circumcincta* larvae were cultured from faecal samples collected daily from five infected donor lambs (see 2.2.4 Parasitology below). Pens were first dosed on a rolling basis over 6 days. Infection was monitored through faecal egg counts every 10 days from the various days of first infection for each pen using the modified flotation method with a sensitivity of one egg per gram (epg) of faeces (Christie & Jackson, 1982). Parasite-induced anorexia was expected in the AP group, so RC lambs were fed the same amount as the AP group voluntarily consumed, based on a 3-day rolling average. This was to control for the confounding effect of anorexia and allow for the assessment of the true impact of parasitism on behaviour and welfare.

Daily feed intake per pen was recorded based on systematic weighing of feed given and leftover feed. Data collection occurred over 4 weeks, from 10 days pre-infection to 23 days post-infection.

2.2.4 Parasitology

Lambs on-farm (but outwith the present trial) were inoculated with *T. circumcincta* to maintain a supply of fresh parasite larvae for the trial. Faeces were collected daily throughout the week using collection bags, then incubated in stable conditions for at least 10 days before the hatched L_3 larvae were collected using the Baermann technique (Walker & Wilson, 1960). This technique involves filtering wet faeces through a funnel to capture the nematode larvae from the sample. The quality and quantity of larvae collected was visually assessed using microscopy, then the larvae were stored in water at 5^{o}C until they were about to be used. Prior to use, the concentration of viable larvae was assessed using microscopy and either concentrated or diluted to ensure that 7,000 viable L_3 would be given within a 3 to 5mL volume of the suspension. The consistency of the larval concentration was checked prior to dosing the trial lambs. Anthelmintic treatment was given to all lambs in the days immediately prior to them being moved into the trial location for a settling-in period, and infected lamb were treated again at the end of the trial.

2.2.5 Data Collection

2.2.5.1 Video recordings

Twelve cameras were placed on posts above 4 pens of each treatment (16 lambs/ treatment) and connected to a computer running GeoVision surveillance software (GeoVision Inc., Taipei, Taiwan). Each camera clearly captured the entirety of one pen. Video was recorded every day for one hour between 13:00 and 14:00 for 28 days. This time slot was selected through analysis of 48 hours of continuous video footage captured one week prior to the beginning of the experiment. Management and experimental procedures were complete by 13:00, meaning the lambs were mostly undisturbed, and the natural light in the barn led to good image quality. Video data were downloaded onto a hard drive every other day and uploaded to an institutional server at the end of the experiment. The functioning and placement of the cameras were checked every morning and they were adjusted as needed.

Behavioural sampling from the videos was conducted by an observer blind to the lambs' treatment groups using The Observer XT 15 (Noldus Information Technology, Wageningen, Netherlands). Three scan samples at 30-minute intervals (minutes 0, 30 and 60 of each video recording) and one 30–minute pen-level continuous behaviour sample were taken from each daily recording, using the ethogram shown in Table 2.1. This number of scan samples was chosen to capture a snapshot of maintenance behaviours in lambs, such as feeding and lying. Scan samples occurred at 13:00, 13:30 and 14:00, while feeding occurred between 9:00 and 10:00 every morning, meaning the first scan was always closest to feeding and there were likely more pellets in the feeders during the first scan compared to the last. A pilot trial compared the prevalence of behaviours performed over 5, 15, 30, 60 and 90 minute focal observation periods to determine how long focal observations should last. No significant differences in the prevalence of play or social behaviour were identified across the observation periods of varied length, therefore 30 minutes of focal observation per day was deemed to be appropriate. Scan samples were carried out at the individual lamb level while behaviour samples were conducted at the pen-level.

Table 2.1.Ethogram of lamb behaviours collected by scan and behaviour sampling for lambs kept in groups of 4 to determine the effects of parasitism on behaviour, where behaviours with an asterisk (*) were used in both scan and behaviour sampling.

Behaviour	Definition
Feeding	Lamb has head within 10 cm of the feed trough, may be seen biting, chewing or obtaining feed.
Drinking	Lamb has head within 10 cm of the water trough, may be seen to be licking, mouthing the trough or obtaining water from trough.
Locomotion	Lamb moves feet, leading to motion in any direction for more than 2 seconds.
Lying	Lamb's body is touching the ground from shoulder to back end, neck and head touching the ground or upright.
Standing	Lamb remains still in a posture where head is raised above the level of the back, up on all four legs.
Pen Exploration	Lamb nudges, noses or chews any object or structure, other than feed, water, bedding or the brush head.
Locomotor play *	Lamb moves rapidly in any direction for more than 2 seconds with no obvious destination to reach, jumping or pivoting for no obvious reason
Social play *	Lamb puts its head down and runs to butt heads with another lamb, or jumps up onto back legs and rests its front half on the back of another lamb
Social behaviour *	Lamb is in any kind of active physical contact with another lamb, including nudging, nuzzling, or nosing. Excludes passively lying close to another lamb and touching it.
Object play *	Lamb's face is within 5 cm of the brush head, or it interacts with the brush head by sniffing, butting, pawing or jumping on it.
Unclear	Lamb's behaviour is concealed by a visual barrier e.g. feeder or another lamb.

2.2.5.2 Qualitative Behaviour Assessment (QBA)

QBA was carried out in person on each pen weekly between 11am and 1pm, a time chosen to avoid disturbances in the barn. The same observer, blind to the lambs' treatment groups, performed QBA every week. After entering or changing positions in the barn, the observer allowed sufficient time for the animals to settle before beginning the observations. For example, if vigilance behaviour began when the observer took their place, observations did not begin until vigilance behaviour disappeared. Once the animals were judged to have resumed their ongoing behaviour, each pen was observed for 2 minutes. This observation duration was chosen based on the instructions to carry out QBA for welfare assessment found in the EU Animal Welfare Indicators (AWIN) project Protocol for Welfare Assessment in Sheep (AWIN, 2015). The list of terms presented in this same document (AWIN, 2015) was used to score the lambs' demeanour using a visual analog scale for every term on a tablet (Xperia S, Sony Europe Ltd., Weybridge, UK). Ninety-six pen-level assessments were carried out over four weeks, with each of the 24 pens being observed 4 times.

2.2.5.3 Weights, Visual Scores and Sampling

All lambs were weighed every 10 days. Before being moved to the weighing area, faecal samples (approx. 6g per animal) were collected in the pen following natural expulsion of faecal matter. If a sufficient faecal sample could not be obtained naturally, a direct faecal sample was collected. Faecal samples were stored in a labelled plastic sample bag for transport to the laboratory, where they were refrigerated at 4^{o}C until egg counting, which was done within 36 hours of sampling. Lambs were then moved to a holding pen linked to a weigh crate. While in the holding pen, faecal soiling score (FSS) and gut fill scores were assigned to every lamb. Faecal soiling was scored on the scale from 0 to 4 developed by AWIN, where:

- 0: No faecal soiling: the wool around the breech area and under the tail is clean
- 1: A small quantity of faecal matter in the wool around the anus
- 2: Some soiling around the anus and dags (matted areas of faecal matter adhering to the wool) in this area only
- 3: Soiling and dags extending beyond the anus to the tail and onto the upper part of the legs
- 4 is Wider area of soiling with dags extending down the legs as far as the hocks.

To record gut fill, lambs were scored as 2 for bloated, 1 for full or 0 for emaciated, as previously described (Phythian et al., 2013a). Lambs were then individually weighed and returned to their home pens.

Faecal egg counts of *T.circumcincta* were conducted by SRUC PhD students. Parasite eggs were counted within 36 hours of faecal sample collection using a salt flotation technique with a sensitivity of 1 egg per gram (epg) of faeces (Jackson and Christie, 1982), and counted on a confocal microscope. Results were entered into Microsoft Excel.

2.2.6 Statistical Analysis

For all analyses, data were separated into pre-infection (from day of infection (DOI) -10 to -1) and post-infection (DOI 0 to 23). The pre-infection dataset was used to determine the baselines of feed intake, behaviour and mental state, while the post-infection dataset showed the effect of infection on these variables.

Scan and behaviour samples were exported from The Observer XT 15 into Microsoft Excel. All statistical analysis was conducted in R 4.2.2 (R Core Team, 2022) via R Studio (version 3.0).

To determine if changes in feed intake took place, a Generalized Linear Mixed Model (GLMM) [glmmTMB package (Brooks et al., 2017)] was utilised using pen as the experimental unit with negative binomial distribution with a quadratic parameterization (nbinom2) link function. Fixed effects included treatment (AC, RC and AP) and day of infection (DOI) as a covariate, as well as the interaction between the two. Pen was included as the random effect.

Behaviours performed more than 5% of the time during scan sampling were analysed. To determine the relationships between the binary behaviours (presence/absence (0,1)) performed during scan sampling and the treatment groups, GLMMs [glmmTMB package (Brooks et al., 2017)] were performed with a binomial probability distribution (binomial) where each lamb acted as the experimental unit. Fixed effects included treatment (AC, RC and AP), scan sample (0, 30 or 60 mins) and day of infection (DOI) as a covariate. Interaction terms included 2-way interactions between *DOI * Treatment*, *DOI * scan*, and *scan * Treatment*. Lamb ID nested within Pen was included as a random effect.

Analysis of data from behaviour sampling included comparisons of total durations and frequencies across treatment groups at pen level (4 lambs combined within pen) for each 30-minute behaviour sample via GLMMs [glmmTMB package (Brooks et al., 2017)]. Social play, locomotory play and object play were combined to form a single play behaviour response variable. The family link function was set to negative binomial distribution with a quadratic parameterization (nbinom2). Fixed effects were DOI and treatment (AC, RC and AP), as well as an interaction (*DOI * Treatment)*. Pen was included as the random effect. Differences in social behaviour and play were compared between the pre-infection and the post-infection period. Negative binomial GLMMs were also used for this analysis where fixed effects included a factor describing the timing of each observation (pre-infection, post-infection) and treatment group, and an interaction term *timing*treatment* was included. Pen was included as the random effect.

Principal Component Analysis (PCA) (Wold et al., 1987) was used to explore differences in lamb affective state across treatment groups as assessed by QBA. A PCA was run on the scores for the descriptive terms (21 total) across observations and pens using the R package *stats*. A scree plot was produced using the package *factoextra* (Kassambra & Mundt, 2020) and the three dimensions that accounted for the highest levels of variance (more than 10%) were retained for graphical representation and modelling. The base R function *print* was applied to the resulting PCA to produce a covariance matrix for the 21 terms and the PCA dimensions. This allowed for interpretation of each dimension. The R package *factoextra* (Kassambra & Mundt, 2020) was used to create graphs of the distribution of pens along the dimensions. It was also used to extract the coordinates of each observation along the first three dimensions. This new dataset contained variables called arousal, valence and aggression, which described the placement of each observation along the respective dimensions. For these three variables, GLMMs were used to evaluate whether the lambs' loadings were related to treatment group or day of infection, with Y+10 to account for negative values in the response variable without disrupting variance. The family link function was set to either negative binomial distribution with a quadratic parameterization (nbinom2) or Gaussian distribution, dependent on model fit and overdispersion parameters (Hardin & Hilbe, 2007). Fixed effects included treatment (AC, RC and RP) and DOI as a covariate, as well as the interaction between the two (*DOI * Treatment)*. Pen was included as the random effect.

A cumulative link mixed model (clmm) [ordinal package (Christensen, 2022) and RVAideMemoire (Hervé, 2023)] with the threshold set to equidistant was used to determine the relationships between FSS and treatment. Model fitness was verified by log-likelihood test in the *ordinal* package (Christensen, 2022). Fixed effects included treatment (AC, RC and RP) and DOI as a covariate, as well as an interaction between the two (*DOI * Treatment)*. Lamb ID was included as the random effect.

For all GLMMs, model fitness was confirmed using the DHARMa package (Hartig, 2022). The ANOVA function in the *car* package (Fox & Weisberg, 2018) was used to determine the significance of explanatory variables based on a $p < 0.05$ threshold and to examine differences between fixed effects and interactions. Pairwise comparisons of estimated marginal means (i.e. adjusted or least-squares means) and associated standard errors were derived with the *emmeans* function of the *emmeans* package (Lenth, 2023) with mode set to "mean.class" to obtain the average probability distributions as probabilities of the visual scores and "response" to obtain estimates of the probability distribution in the response scale for each treatment group, with Tukey adjustment of p-values accounting for multiplicity. *Emmeans* (Lenth, 2023) was also used to examine linear trends between fixed effects and covariates. Graphical representations of results were produced using *ggplot2* (Wickham, 2016) with corrected pairwise comparisons with standard error (SE) and 95% confidence intervals (CIs) reported.

2.3 Results

2.3.1 Pre-infection results

Feed intake was significantly lower for RC lambs between DOI -10 and -1 than for AC and AP lambs ($p<0.001$), when all animals were being fed ad libitum. Treatment group did not have a significant effect on any of the behaviours studied during scan or behaviour sampling in the pre-infection period. Scores from the QBA were similar across all pens. Loadings along the arousal dimension increased for all treatments across the pre-infection period, although there was a significant difference in the rate of that increase between AP and RC ($p=0.007$), where arousal loadings for AP lambs increased at a slower rate than other treatment groups. There was a significant effect of treatment group on FSS in the pre-infection period, where RC lambs (1.99±0.16) had higher FSS than AC (1.86±0.15) and AP lambs (1.70±0.16), who had the lowest scores ($X_{2(2,29)}$= 40.24, p<0.001).

2.3.2 Post infection Results

2.3.2.1 Faecal Egg Counts (FEC)

The parasitised treatment group (AP) was the only group whose FEC rose above zero for the entire study period, and only from DOI 11. That day, AP lambs began showing low FECs of 1.4 ± 0.6 (mean ± SE) epg. On DOI 12, AP lambs had a mean FEC of 3.2 ± 0.7. Ten days later, on DOI 21, all 32 AP lambs were shedding eggs, with a mean FEC of 77.2 ± 14.7, and AC and RC lambs' FEC remained at 0. A Kruskal-Wallis test of FEC on DOI 21, the first day of the patent period of infection when lambs are expected to start shedding parasite eggs, found a significant difference between the parasitised lambs and lambs in the two control groups ($X_{2(2)}$=90, p<0.001).

2.3.2.2 Feed Intake

Feed intake increased over time for all three treatment groups as the lambs grew. Mean feed intake during the infection period for AC lambs was 10213g/day ± 72.9, 9585g/day ± 54.0 for RC lambs and 10059g/day ± 70.3 for AP lambs. There was a significant effect of the interaction between DOI and treatment group on feed intake (p=0.003). The increase in feed intake over time for AC lambs was significantly greater than for AP lambs (p=0.002). There was no significant difference in feed intake over DOI between RC and AC or AP lambs. Detailed feed intake results based on the systematic feeding of feed given and feed leftover are to be reported in a future PhD thesis by Naomi J. Booth entitled *Quantifying the effects of parasitism on livestock greenhouse gas emissions*.

2.3.2.3 Scan Samples

The most frequently recorded behaviour was lying, and the least frequently observed was object play. Lying, standing and eating were the most frequently observed behaviours, accounting for 61.6, 15.1 and 16.4% of observations respectively. The other behaviours in the ethogram (Table 2.1) were seen less than 5% of the time, and therefore were not analysed.

i. Lying behaviour

Scan number had a significant effect on lying behaviour. Lying was less likely to occur during scan 1 (prob=0.48, SE=0.02) than scan 2 (prob=0.68, SE=0.02)(OR=0.42, SE=0.04, z= -8.11, p<0.001) and scan 3 (prob=0.70, SE=0.02)(OR=0.40, SE=0.04, z= -8.64, p<0.001). There was no significant effect of treatment group on lying behaviour and no significant interaction between DOI and treatment group.

ii. Standing behaviour

When modelling standing behaviour, there was a significant interaction between DOI and treatment group (X^2=9.55, df=2, p=0.008). As shown in Figure 2.1, AP lambs were more likely to be standing as DOI increased (est=0.10, SE=0.02) than AC lambs (est=0.02, SE=0.02)(p=0.006). The RC lambs' likelihood of standing did not differ from either group (Figure 2.1).

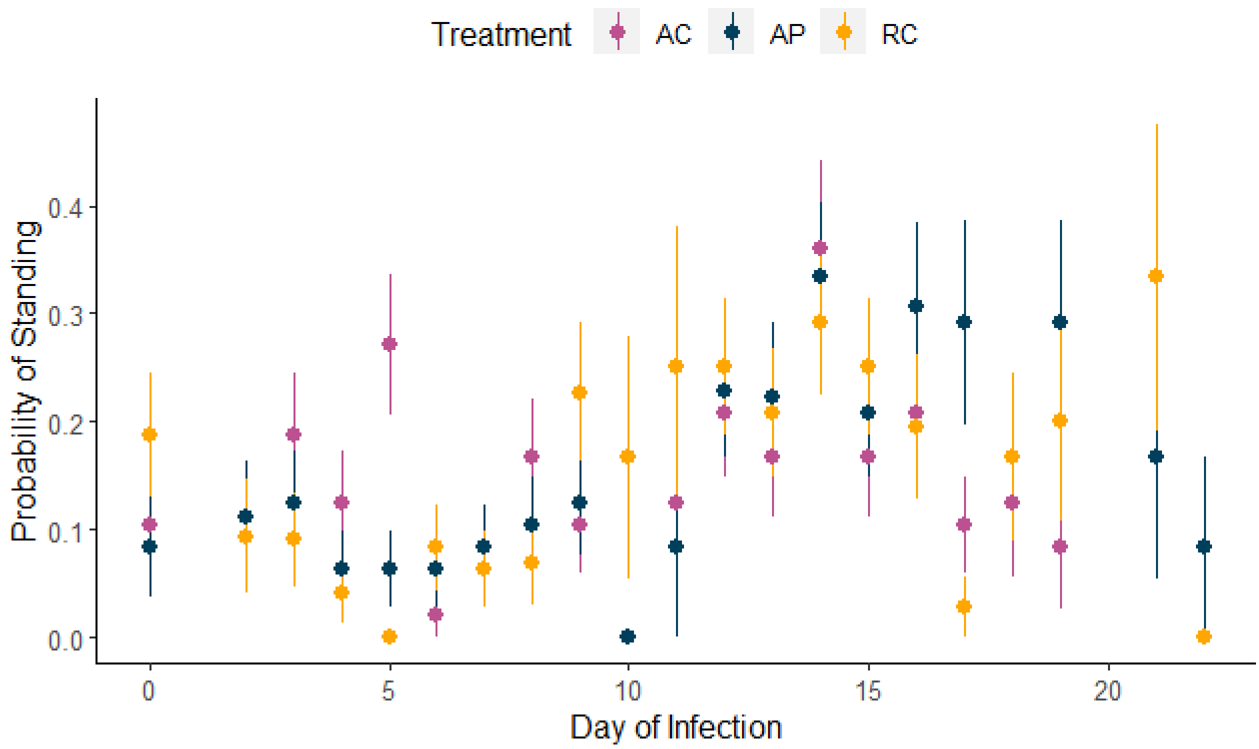

Figure 2.1. Mean probability of standing behaviour by treatment group from day 0 of infection to day 23 of infection, where AC=ad-lib fed control, RC=restricted-fed control and AP=ad-lib fed parasitised.

There was a significant interaction between treatment group and scan number for standing behaviour (X^2=23.47, df=4, p<0.001). Lambs in the AC group showed a significant decrease in likelihood of standing behaviour between scans 1 and 3 (p=0.003), while RC's decreased between scans 1 and 2 (p<0.001) and scans 1 and 3 (p<0.001). Lambs in the AP group lambs showed no significant difference in standing behaviour over scans, meaning they were equally likely to be standing across the entire scan sampling period.

iii. Eating behaviour

There was a significant interaction between treatment group and scan number for eating behaviour (X^2=18.54, df=4, p<0.001). During scans 1 and 2, there were no significant differences between treatment groups. However, during scan 3, AP lambs

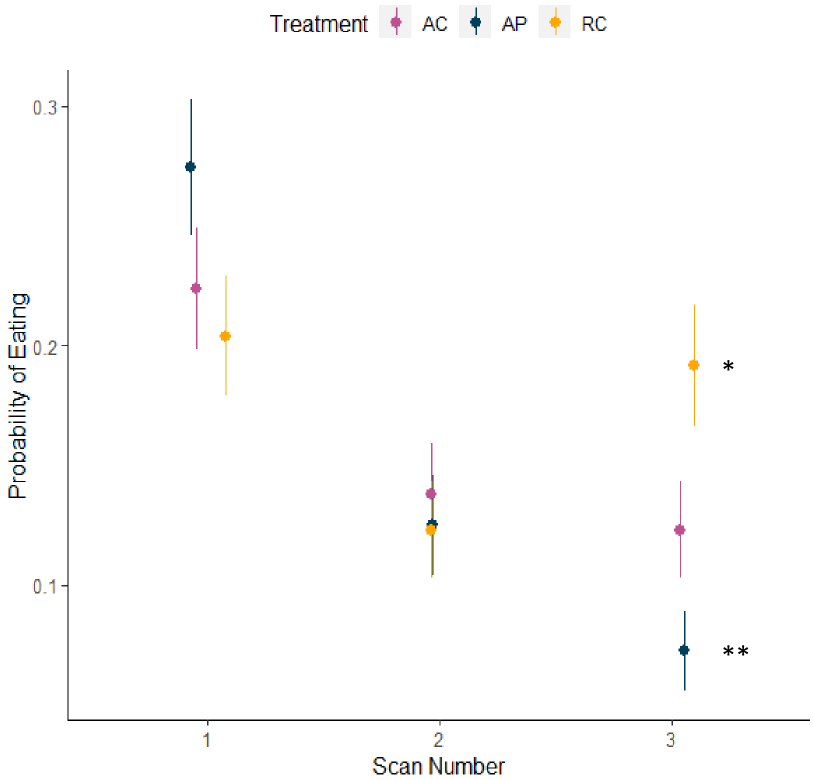

Figure 2.2. Mean probability of eating behaviour across the three scan samples by treatment group, where AC=ad-lib fed control, RC=restricted-fed control and AP=ad-lib fed parasitised. Dots with differing star symbols are significantly different from each other.

were significantly less likely than RC lambs to be performing eating behaviour (OR=0.32, SE=0.10, z= -3.74, p<0.001) (Figure 2.2).

2.3.2.4 Behaviour samples

Lambs performed social behaviour and play behaviour 295 and 45 times respectively (Table 2.2).

Table 2.2. Total number of bouts, total duration of bouts, and mean duration of bouts for social behaviour and play at the pen level for the infection period across treatment groups.

	Treatment Group	**Play**	**Social Behaviour**
Total number of bouts	AC	21	94
	RC	10	81
	AP	14	120
	Total	**45**	**295**
Total duration of bouts (s)	AC	830.6	856.8
	RC	1279.4	1057.6
	AP	328.3	1244.6
	Total	**2438.3**	**3159.0**
Mean ± SE duration of bouts (s)	AC	2.5 ± 1.3	3.0 ± 0.6
	RC	12.5 ± 6.5	6.8 ± 2.0
	AP	3.2 ± 1.1	4.4 ± 0.8
	Mean	**6.0** ± 1.8	**4.7** ± 1.8

There was a significant interaction between DOI and treatment group when modelling total duration of play ($X_{(2)}$=6.13, p=0.047). Play bout duration decreased over time for AC (est=-0.13, SE=0.07) and AP (est= -0.06, SE=0.08) lambs but increased for RC lambs (est=0.12, SE=0.08)(Figure 2.3). There were no significant effects of DOI or treatment group on total duration of social behaviour or on the number of bouts of social behaviour and play performed by each pen.

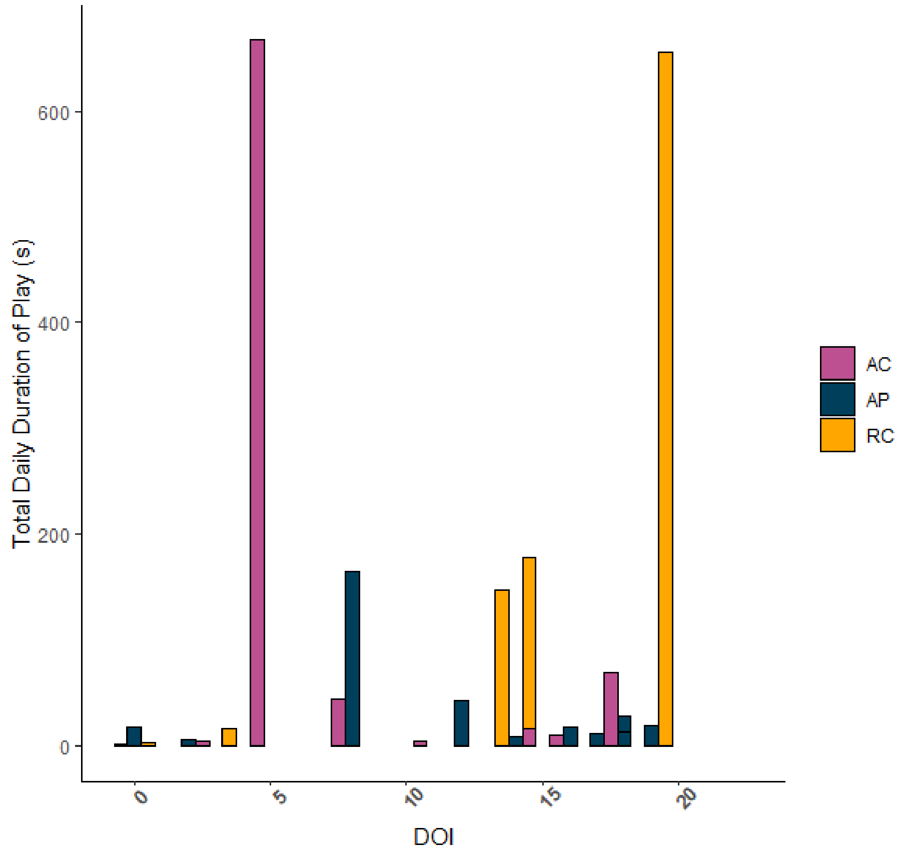

Figure 2.3. Total daily duration of play behaviour over day of infection for the three treatment groups, where AC=ad-lib fed control, AP=parasitised and RC=restricted-fed control lambs.

When comparing before and after infection, there was a significant decrease in the number of social behaviour bouts after infection for all treatment groups (OR=0.45, SE=0.11, z = -3.41, p<0.001).

2.3.2.5 QBA

The PCA revealed that principal component 1 (PC1) accounted for 36.7% of the variance, PC2 accounted for 15.1% of the variance, and PC3 accounted for 12.8% of the variance. Cumulatively, PC1, PC2 and PC3 accounted for 64.6% of the variance in the QBA data.

PC1 described arousal levels, with terms such as \`Calm\`, \`Relaxed\`, and \`Subdued\` on one end and \`Active\`, \`Vigorous\` and \`Assertive\` on the other. I interpreted that PC2 described the valence of the animals' affective states, running from \`Agitated\`, \`Apathetic\` and \`Physically Uncomfortable\` to \`Content\` and \`Bright\`. I interpreted that

PC3 described the spectrum of fear and aggression, running from `Sociable` and `Aggressive` to `Alert`, `Fearful`, and `Tense` (Table 2.3).

Table 2.3. Matrix of the 21 QBA terms for pen-level observations. Blue cells show the two terms with the highest positive values and orange cells show the two lowest negative values.

Term	PC1	PC2	PC3
Alert	-0.1207	-0.2635	-0.2807
Active	-0.2933	-0.0123	0.2037
Relaxed	0.2728	-0.2555	-0.0638
Fearful	-0.1132	0.1860	-0.4681
Content	0.1555	-0.4150	-0.0330
Agitated	-0.1220	0.3182	-0.0968
Sociable	-0.2061	-0.0754	0.2383
Aggressive	-0.1940	0.1129	0.2099
Vigorous	-0.3153	-0.0598	0.1383
Subdued	0.2837	0.2012	0.1028
Physically uncomfortable	0.0742	0.2876	-0.0218
Defensive	-0.1671	0.0629	0.1963
Calm	0.3206	-0.1458	-0.1049
Frustrated	-0.1108	0.2482	-0.0460
Apathetic	0.2560	0.2836	0.1723
Wary	-0.0963	0.0887	-0.4704
Tense	-0.1427	0.2567	-0.3883
Bright	-0.2525	-0.3055	-0.0996
Inquisitive	-0.2703	0.0189	0.0482
Assertive	-0.3010	0.0106	0.1375
Listless	0.2038	0.2766	0.1735

Treatment group had no significant impact on the distribution of pens along PC1 or PC2 over the infection period of the experiment. However, loadings along PC3, the dimension describing aggression and fear, were different across treatment groups. Lambs in the AP group had lower loadings on PC3 (est=9.42, SE=0.33) than RC lambs (est=10.64, SE=0.33), meaning they were behaving more fearfully than RC lambs (p=0.030). AC's loadings on PC3 were not significantly different from either RC or AP (Figure 2.4).

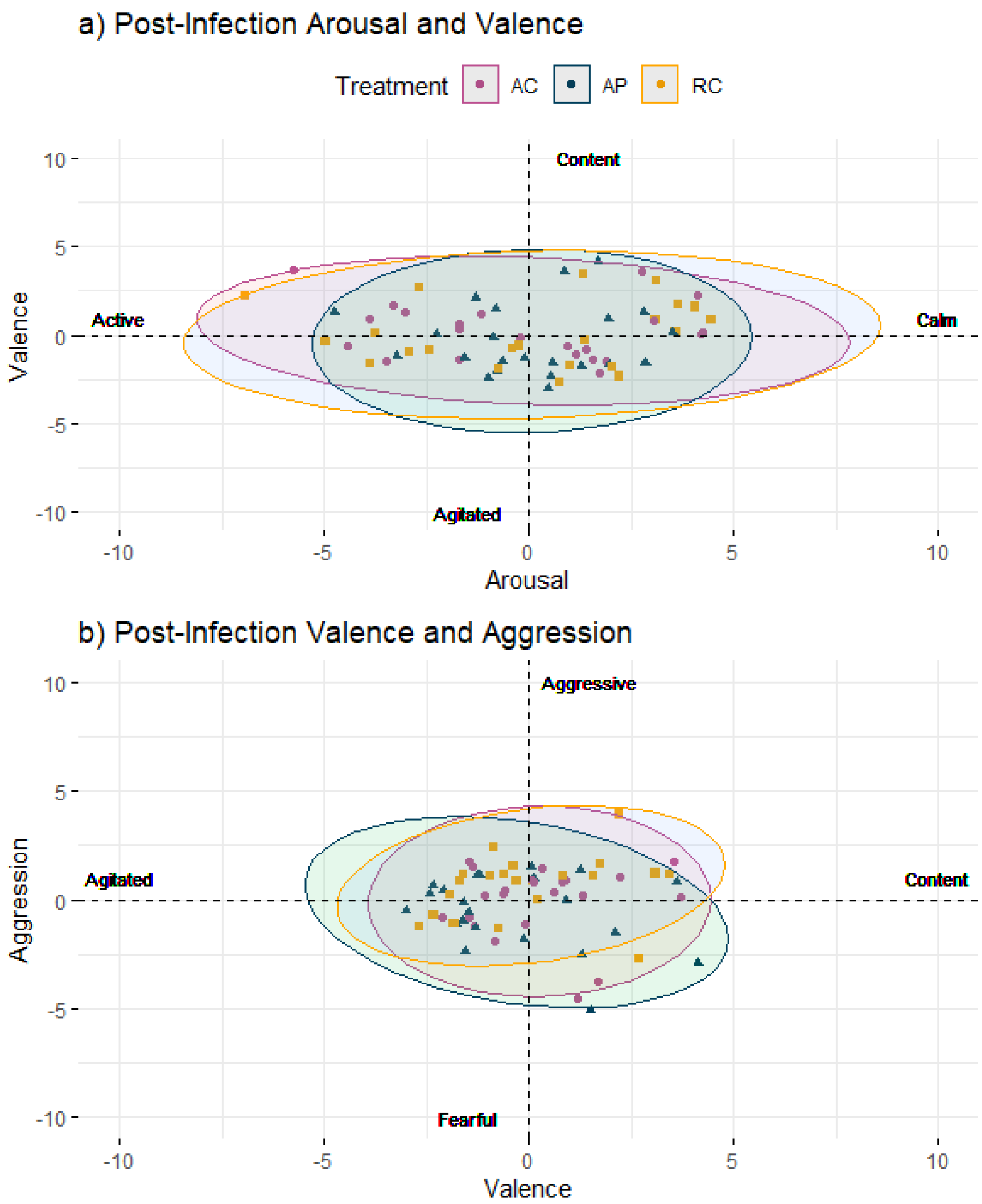

Figure 2.4. Plots of pens over the infection period with a) PC1 (arousal) on the x axis and PC2 (Valence) on the y axis and b) PC2 (Valence) on the x axis and PC3 (Aggression) on the y axis. Terms at both ends of the axes are anchors for the principal components. AC=ad-lib fed control, AP=parasitised and RC=restricted-fed control lambs.

2.3.2.6 Visual scores

i. Gut fill

All lambs scored a gut fill of 1 (normal fill) at every sampling day throughout the study.

ii. Faecal soiling scores (FSS)

For all treatment groups during the infection period, FSS 1 was most often recorded, and FSS 4 was only recorded 5 times. The AC group had a median FSS of 3 (IQR=2), RC lambs' median FSS was 2 (IQR=1) and AP lambs' median FSS was 2 (IQR=1). The FSS of all treatment groups increased over time. There was no significant effect of treatment group on FSS. None of the behaviours recorded during scan sampling and included in the model (lying, standing and eating) or behaviour sampling (social behaviour, play) had a significant relationship with FSS.

2.4 Discussion

The aim of the study was to identify early animal-based indicators of GI parasitism and to understand the welfare impact that the disease has on lambs. Subclinically parasitised lambs were more likely to stand and less likely to display eating behaviour than unparasitised control lambs. Furthermore, QBA found that they scored higher on terms related to fear than non-parasitised lambs.

T.circumcincta egg counts were low, as the study period only extended 23 days after infection, thus capturing the prepatent phase of infection and the beginning of the patent phase. The patent phase is when egg shedding begins, between 17 and 21 days after infection (Wood et al., 1995). The maximum FEC reached by AP lambs in this trial was 360 epg on day 21. It is likely that only part of the full range of potential behavioural changes occurring during subclinical infections were captured. However, behavioural changes have been seen in the prepatent phase in previous studies. Morris *et al*. (2022) found that parasitised lambs on pasture had a lower motion index and step count and fewer lying bouts, compared to control lambs in the prepatent phase.

While feed intake increased over the entire study period for all treatment groups, AP lambs had a smaller increase over time than AC lambs. The RC lambs had a much lower mean intake than AP lambs, especially pre-infection and in the first 5 days of infection. The reason behind this lower intake is unknown. An unplanned outbreak of *Yersinia pseudotuberculosis* affected most of the lambs in the trial as of day 14 of the experiment (Day of infection 5, 7, 10, and 12 for different groups). For all treatment groups, this caused feed intake to decrease sharply and faecal soiling seemed to increase. This infection may have affected the RC lambs' feed intake, and indeed all of the lambs' behaviour. The original purpose of the RC group was to separate any behavioural and welfare impacts of hunger from those of parasite infection. This separation was rendered impossible by the RC pens seemingly eating to satiation despite their restriction. The RC treatment effectively acted as a second control group instead. However, the reduced increase in feed intake over time seen in AP lambs was significantly different from the pattern in AC lambs, and likely reflects the onset of parasite-induced anorexia. This has been reported during subclinical infection in the past, sometimes accounting for a reduction of up to 20% in feed intake (Laurenson et al., 2011).

Some differences in lying, standing and eating behaviour across the three scan samples likely reflects the lambs' daily routine; they were fed between 9:00 and 11:00 every morning, and scans 1, 2 and 3 occurred at 13:00, 13:30 and 14:00PM respectively. This means that the first scan was always closest to feeding and there was likely more pellets in the feeders during the first scan compared to the last. The decreased likelihood of lying during scan 1 reflects the increased likelihood that the lambs were still standing and eating.

The increase in standing behaviour for AP lambs was mostly seen between day 5 and 20 post-infection. In this study's ethogram, behaviour categories were mutually exclusive, therefore standing can be considered an inactive behaviour. These results could therefore reflect previous findings where activity in many species was reduced during a health challenge (Gauly et al., 2007; Ghai et al., 2015; Hart, 1988; Morris et al., 2022). In the present study, parasitised sheep possibly stood more due to abdominal pain caused by the abomasal damage being inflicted by the parasitic larvae, although the exact reason for the increase cannot be confirmed. This result leads us to accept our hypothesis that parasitised lambs reduced their activity levels compared to uninfected lambs.

Some differences in likelihood of eating behaviour across treatment group may result from the onset of anorexia in the prepatent phase of parasitism in AP lambs. The likelihood of observing eating behaviour remained low after scan 1 for AP lambs, whereas control lambs were just as likely to be eating during other scans. This could reflect the findings of previous studies reporting reduced feeding bouts in parasitised ruminants (Fox et al., 2013; Hutchings et al., 2000, 2002). We can accept the hypothesis that parasitised lambs reduced their feeding activity.

Reduced play and socialising are recognized components of sickness behaviour in many mammalian and avian species (Dantzer & Kelley, 2007; Hart & Hart, 2019; Johnson, 2002; Weary et al., 2009). Notably, when parasitised with *T.circumcincta*, it has been reported that lambs reduced the frequency of contact with conspecifics (Morris et al., 2022). The reduction in social behaviour after infection was seen across all treatment groups in this study, including non-infected controls. We rejected our initial hypothesis that only parasitised lambs would reduce their social behaviour. Interactions between lambs could have decreased over time as the lambs aged and became accustomed to their surroundings. Social interactions are subject to breed differences, with English lowland breeds and Scottish hill breeds, such as the ones in this trial, being some of the least gregarious (Dwyer & Lawrence, 1999). It is possible that 30-minute daily behaviour samples were not long or frequent enough to capture much social behaviour given the breed of sheep being studied. Play is influenced by the environment, as shown by the difference in frequency of play in desert and mountain wild sheep (Berger, 1979). The indoor pens used here were relatively bare, so space for play and social interaction may have acted as a limiting factor (Berger, 1979). RC lambs' play bout duration may have increased over the post-infection phase because they were a particularly playful or aggressive group of lambs, as shown through their non-significantly higher aggression loadings in QBA pre-infection and significantly higher aggression loading post-infection (although RC lambs were not infected themselves). It was not possible to differentiate between antagonistic and playful bouts of head-butting and jumping during observations, so it is unclear if the RC lambs were truly more aggressive, or if they were simply more playful lambs.

The GLMMs used to analyse lying, standing and eating behaviour met the assumption of linear residuals, but the dispersion of the residuals was not entirely homogenous. This is likely due to a number of sources of variation in the data that were unaccounted for during data collection, and therefore not included in the models. This limitation is taken into account when interpreting the results of the models.

The PC3 axis described a spectrum of behaviour ranging from freezing and remaining alert, to engaging in antagonistic social interactions in reaction to potential danger. Post-infection, AP lambs' behaviour was characterised by this alert and tense freezing response, differing from RC lambs who had higher loadings on the aggression side of the axis. This reflects non-significant results in the pre-infection period where RC lambs also had higher aggression loadings than AP lambs. It is possible that sick prey animals would increase their vigilance behaviour, as they are more vulnerable to predators. Young (2006) found that lambs who were experiencing pain showed more vigilant behaviour in the presence of predators. In their study of sheep using a walk-over-weigh system on pasture, Grant *et al.* (2018) found that observers described inappetent sheep as more `reluctant`, `tense` and `wary` than control sheep, although the reason for their inappetence was not reported. These findings suggest that qualitative assessments of behavioural expression could contribute to identifying GI parasitism in sheep. They also suggest that infection, separate from the effect of the anorexia it brings on, has a welfare impact on lambs by potentially increasing fear and anxiety levels. This leads us to accept the hypothesis that parasitised lambs experienced a negative mental state.

The lack of variation in gut fill scores may be due to the score being too crude to account for minor differences between lambs, and only detecting very significant welfare impacts. This score was found by Phythian *et al*. (2013) to be useful as part of a wider welfare assessment index due to its good inter-observer agreement. Rumen fill is often used in cattle studies but rarely appears in sheep trials (Zufferey et al., 2021). Its use did not lead to any conclusions in this study, therefore we must reject the hypothesis that parasitism would cause lower gut fill scores.

In this experiment, FSS was not a good predictor of FEC. In one study, FSS has been reported to have a low to moderate positive phenotypic correlation with FEC (Bisset et al., 1992). On the contrary, in their work on creating low and high FEC lines of Romney sheep, Morris *et al.* (2000, 2005) found an increased FSS in the low FEC line. Pollott *et al.* (2004) found low genetic correlations between FEC and FSS in Merino sheep. They concluded that while it is an indicator of scouring, it is very different from FEC as an indicator of infection (Pollott et al., 2004). This reflects our finding of no treatment effect on faecal soiling, which leads us to reject our initial hypothesis that parasitised lambs would have higher FSS.

2.5 Conclusion

Early indicators of disease are crucial to encouraging prompt treatment of health issues in extensively farmed sheep and lessening their impact on animal welfare. We demonstrated that subclinically parasitised lambs increased standing behaviour and decreased eating behaviour over time compared to non-parasitised lambs. These changes have the potential to act as early indicators of GI parasite infection. If behaviour can be monitored remotely in extensively farmed sheep, infection could be detected early and at the individual level without gathering the flock. The QBA results suggest that parasitised lambs experienced more negative affective states linked to fear and anxiety compared to non-parasitised lambs. This finding contributes to the small body of evidence that GI parasitism, even at a subclinical level, negatively impacts lamb welfare not only in the health domain but in the behaviour and mental domains as well.

Chapter 3. Behaviour as an early-warning system for compromised welfare in extensively farmed sheep

3.1 Introduction

Sheep raised in extensive conditions face varying welfare challenges, including often undiagnosed and untreated disease and injury, inappropriate or variable nutrition, predation, thermal stress and neonatal mortality (Dwyer et al., 2021; Munoz et al., 2018; Rioja-Lang et al., 2020; Rioja-Lang et al., 2020a). Lameness, parasitism and mastitis are among the principal welfare challenges for sheep in European extensive production systems (Czizster et al., 2022; Dwyer et al., 2021; EFSA, 2014; Sossidou et al., 2021). They are therefore the welfare conditions studied in the experiment reported here. Applying the principles of the Five Domains, all three conditions negatively impact the health domain and could have negative affective consequences on the mental domain (Mellor & Beausoleil, 2015). They could also have a negative, indirect impact on the sheep's agency to express behaviour (the fourth domain), for example through a lame animal being unable to graze due to its condition. This means they have important welfare implications, and it is important to detect and treat them as early as possible, thereby alleviating the negative affective states in the mental domain (Mellor & Beausoleil, 2015). These infections trigger an immune response from the sheep, which produces pro-inflammatory cytokines, and cause behavioural changes such as anorexia, lethargy and decreased social motivation (Hart, 1988; Nordgreen et al., 2020). These changes, referred to as the sickness response, are an adaptation allowing the animal to divert resources to the immune response in order to recover (Hart, 1988; Nordgreen et al., 2020).

Early indicators of lameness are crucial because early detection and treatment are key to reducing its prevalence (Kaler et al. 2020). For this reason, identifying the sickness behaviours associated with the onset of lameness could help identify lame sheep earlier. Studies in cattle have found that lame cows spend more time lying in fewer, longer bouts, have lower body condition scores (BCS) and reduce their pedometric activity by at least 15% (Barwick et al., 2018; Green et al., 2014; Westin et al., 2016). Changes in walking behaviour and gait are the principal ways in which lame sheep are identified. However, the trend towards reduced numbers of stockpeople on farms makes visual observation difficult (Barwick et al. 2018). Additionally, because sheep are prey animals, they often hide signs of lameness from humans (Kaler et al. 2020).

Behavioural effects of gastrointestinal (GI) parasite infection in sheep include anorexia and changes in diet selection (Jones et al. 2006; Fox et al. 2013). Anorexia is both a result of and a response to parasitism (Kyriazakis 2014). The reduction in feed intake is part of the animal's immune response as well as the results of abomasal damage brought on by GI parasites (Coop et al., 1985; Greer et al., 2008; Kyriazakis, 2014). The extent of anorexia is thought to increase with greater larval challenges (Laurenson et al. 2011). Studies have found that voluntary feed intake in lambs began to decline during the fourth week following infection (Kyriazakis et al., 1996). This suggests that the presence of established adult worms is needed for inappetence to develop (Kyriazakis et al., 1996). The lower herbage intake of parasitised sheep is due to shorter grazing bouts than non-parasitised sheep (Hutchings et al. 2000). The results of the study in Chapter 2 of this thesis were similar whereby parasitised animals were less likely to be observed performing feeding behaviour. Reduction in activity levels in parasitised sheep have been reported, which was moderated when they were mixed with non-parasitised sheep on pasture (Morris et al., 2022). As described in Chapter 2, this inactivity may come in the form of increased incidence of standing inactive behaviour. Therefore, measures of appetite such as feed intake and activity could act as indicators of GI parasite infection.

While clinical mastitis is a widely acknowledged welfare issue in sheep flocks in the UK, the effect of subclinical mastitis on sheep welfare is less clear (EFSA, 2014; Rioja-Lang et al., 2020a). It is more prevalent than its clinical form in most dairy animal populations, accounting for up to 95% of mastitis cases in sheep (Martins et al. 2013; Sinha et al. 2018). Diagnosis relies on milk-based measures, such as somatic cell count (SCC) or the California Mastitis Test (CMT) (Conington et al. 2008; Sinha et al. 2018), which are difficult to obtain in meat sheep. Studies such as one applying automated pain facial expression detection systems in ewes suggest mastitis is a painful condition (McLennan et al., 2016). Pain and discomfort are two factors that negatively impact animals' affective experience domain (Mellor, 2017). There is a need to identify practical indicators of subclinical mastitis (Sinha et al. 2018) and to determine its impact on meat sheep welfare in extensive systems. When assessing welfare, some researchers suggest it is important to consider the risks of future welfare compromise as well as the actual welfare concerns, especially in extensive systems (Richmond et al., 2017). Subclinical cases of mastitis can lead to clinical cases, therefore they could be classified as welfare concerns (Arsenault et al., 2008; Watkins et al., 1991). There are few practical animal-based indicators of subclinical mastitis. In dairy breeds, research suggests that a reduction in lamb sucking behaviour could be a behavioural indicator of subclinical mastitis in ewes (Gougoulis et al., 2008). Having three or more lambs and being multiparous are risk factors that increase the odds of subclinical mastitis in meat ewes (Arsenault et al., 2008; Lafi et al., 1998; Watkins et al., 1991).

Measuring the behavioural changes that occur during the sickness response may be a way of identifying welfare concerns before clinical signs of disease are obvious and the animal's welfare is further compromised. Understanding these behavioural changes may allow us to monitor them remotely using technology in the future. The economic benefits of using Precision Livestock Farming (PLF) technology to monitor health and production are often discussed in the literature; Electronic Identification (EID) paired with electronic weighing and drafting in a mountain sheep system resulted in a 36% decrease in labour required for anthelmintic treatments and an increased net profit margin on the farm (Morgan-Davies et al., 2018). In an economic modelling study, the application of automatic heat detection on a large dairy farm was found to lead to over €7000 in increased profit (Kamphuis et al., 2015) and machine vision with deep learning models can monitor feed intake for individual cows, allowing for improved feed efficiency (Saar et al., 2022). But there is an opportunity to centre the development of PLF for small ruminants, a much less developed PLF field than for cattle, around welfare management (Morrone et al., 2022). Technology based on accelerometers or Global Positioning Systems (GPS) that track and interpret animal behaviour could provide a wealth of information on welfare that was previously unavailable (Morrone et al., 2022). For example, in cattle, rumination and activity sensors successfully identified cows diagnosed with metritis and ketosis after calving (Steensels et al., 2017), and changes in feed behaviour measured by automatic feeders characterised the onset of ketosis and acute locomotion disorders (González et al., 2008). For this to become a reality in sheep farming, the early behavioural expressions of welfare concerns must be understood and identified and return-on-investment for farmers must be ensured (Odintsov Vaintrub et al., 2021).

This chapter covers two trials that were carried out on the same lowland farm over the course of two grazing seasons in 2021 and 2022. The first was a pilot trial carried out from June to October 2021 and the second was a larger, similar trial from April to September 2022. Their aim was to measure the behavioural changes that occur during the onset of lameness, mastitis and GI parasitism in lambs and ewes in extensive systems. I hypothesised that sheep experiencing these welfare challenges would have reduced overall activity, including social and feeding activity. They would spend more time lying and less time grazing, walking and performing social behaviour than non-infected sheep. I also hypothesised that these behavioural changes would occur before any clinical symptoms became apparent.

3.2 Methods

3.2.1 Ethical approval

Ethical approval was obtained from the Moredun Research Institute's AWERB (Animal Welfare and Ethical Review Board (Trial 20-21 and Trial 18-22, project number PPL P95890EC1I).

3.2.2 Animals and Management

In both years, Scottish Mule ewes and their twin lambs were studied at Firth Mains Farm in Midlothian, Scotland. In 2021, ewes of varied age and parity were used whereas in 2022, all ewes were 2 years old and primiparous. All lambs had tails docked and males were castrated. The animals were housed indoors during lambing then turned out onto paddocks within 36 hours of lambing, as per usual farm practice. All paddocks on which the experiments ran over both years were originally sown with a mix of 60% perennial rye grass, 10% timothy and cocksfoot and 5% white and red clover and had been grazed by sheep for several years prior (Barrett, 1997). They were known to contain *Nematodirus* and strongyle species, including an isolate of *Teladorsagia spp.* resistant to drugs within the Bz class of anthelmintics (Barrett, 1997).

3.2.2.1 Animals in 2021

Twenty-four ewes and their 48 twin lambs were studied. Behavioural data collection was carried out over two 4-week periods, coinciding with known GI parasitism peaks, from June 7th to July 5th 2021 (Phase 1 (P1)-2021) and September 6th to October 1st 2021 (Phase 2 (P2)-2021). The ewes and lambs grazed together during P1-2021 on three adjoining 1-hectare paddocks. The gates separating the paddocks were left open to allow free movement between them. The rectangular paddocks had trees and large dead branches at one end which provided shade. At the opposite end, there was a drinker in each paddock. During P2-2021, only the lambs were observed and remained on the paddocks, as the ewes were not studied following weaning on August 9th, 2021, when lambs were 18 weeks of age.

3.2.2.2 Animals in 2022

Thirty-six ewes and their 72 twin lambs were used. Behavioural data collection was carried out over four observation periods lasting 3 to 4 weeks and coinciding with known welfare challenging events (e.g. weaning, *Nematodirus* egg shedding peak). Observations ran from April 11th to 28th 2022 (P1-2022), May 10th to June 10th 2022 (P2-2022), July 18th to August 12th 2022 (P3-2022), and August 30th to September 23rd 2022 (P4-2022). Ewes and lambs were observed together during P1-2022, P2-2022 and the first two weeks of P3-2022, after which the lambs were weaned at 18 weeks of age on August 2nd, 2022. Ewes and lambs were observed separately during the last two weeks of P3-2022 and only the lambs were observed during P4-2022.

3.2.3 Experimental Design

3.2.3.1 Experimental design in 2021

Each ewe and her two lambs acted as one replicate. The 24 ewes and their lambs were divided into two treatment groups (n=12). Half of the animals were treated orally with an anthelmintic drench, following the manufacturer’s recommended dose rate of 2.5ml per 10kg of body weight (Oramec, Boehringer Ingelheim International GmbH, Ingelheim, Germany) to act as a “low parasitism” group (LP). Sheep in the LP group were treated monthly in order to implement a suppressive worming strategy (Kenyon et al., 2013). The other half were only treated if they met criteria indicative of a clinical level of infection and acted as a “high parasitism” group (HP). Sheep in the HP group were treated if they had a faecal egg count (FEC) above 800 epg (strongyle or *Nematodirus*), lost 10% of their previously recorded weight, or had a dag score of 3 or above (see Table 3.1). Both groups were exposed to naturally occurring GI infection. Within the LP and HP groups, half of the ewes and one lamb from each set of twins wore collars containing technological tools. The other half did not wear collars to act as a control group to allow us to study the effect of wearing the collars on behaviour and welfare. All results related to the impact of wearing collars are reported in Chapter 4 of this thesis. Ewe and lamb allocation to parasitism and collar treatment groups was randomized and balanced for ewe weight, mastitis score and FEC at the start of the study, as well as lamb sex.

3.2.3.2 Experimental design in 2022

The parasitism drench treatment was not repeated in 2022, as only lamb treatment groups had a significant difference in only strongyle FEC in 2021 (see Results section). Every ewe and her lambs acted as an experimental unit (n=36) and were exposed to naturally occurring infection. Individuals were only treated if they met criteria indicative of a clinical level of infection, defined as having a FEC above 800 epg (strongyle or *Nematodirus*), losing 10% of their previously recorded weight, or having a dag score of 3 or above (see Table 3.1). If one of these criteria was met, sheep were treated with anthelmintic drench, following the manufacturer's recommended dose rate of 2.5ml per 10kg of body weight (Oramec, Boehringer Ingelheim International GmbH, Ingelheim, Germany). The technology treatment groups were repeated, with 18 ewes and one lamb from each pair of twins wearing a collar.

3.2.4 Data Collection

3.2.4.1 Sampling and Scoring

i. Data collected in 2021

Before sheep were turned out onto the paddocks for the first time after lambing, they were labelled with a number from 1 to 12 in red or green livestock paint (Ritchey Livestock ID Ltd., Brighton, USA) for easy visual recognition of individuals. Each ewe and her lambs were sprayed with the same colour and number, with the twins differentiated by the presence of a large dot between one twin's shoulders. All ewes and lambs were weighed fortnightly using a Combi Clamp weigh crate (Ritchie UK Ltd., Fofar, UK) and Tru-test XR5000 weighhead (Tru-test Group, Auckland, New Zealand) ten metres from the paddocks where the sheep grazed. While the sheep were in the weigh crate, a faecal sample between 1 and 5 grams was collected per rectum by a trained technician and stored in a labelled plastic sample bag for transport to the laboratory, where they were refrigerated at 4^{o}C until egg counting. While still in the weigh create, every lamb and ewe was assigned a dag score and ewes were assigned a body condition score (BCS) and a mastitis score by the same observer throughout the study. A BCS by palpation (from 1-Emaciated to 4-Fat), faecal soiling (from 0-Not present to 4-Extensive soiling and dags) and mastitis (from 0-No mastitis or lesions present to 2-Mastitis and/or severe lesions) were scored on the scales developed by AWIN (AWIN, 2015). Once a month, the treatment groups receiving anthelmintic treatment were drenched while in the weigh crate as described above. Visual ID numbers were re-painted on a monthly basis to allow for continuous clear identification in the field. Faecal samples were processed to determine the number of strongyle and *Nematodirus* parasitic worm eggs present. Parasite eggs were counted within 72 hours of faecal sample collection at the Moredun Research Institute using a salt flotation technique with a sensitivity of 1 egg per gram (epg) of faeces (Jackson and Christie, 1982), and counted on a confocal microscope. Results were entered into Microsoft Excel.

ii. Data collected in 2022

Sheep were labelled for the first time in the same way as 2021 with numbers from 1 to 18 in red or green livestock paint before turnout after lambing. Gathering, weighing and sampling occurred fortnightly as in 2021 in the same handling facility and while animals were being held in the weigh crate. However, the mastitis scoring scale was adjusted to include more categories after experience from 2021 and discussion with the wider TechCare team conducting simultaneous pilot trials (Table 3.1). The new welfare scoring scales were specifically designed by the TechCare team to harmonise welfare assessments across all experimental trials being undertaken in the TechCare project and were based on the AWIN Welfare Assessment Protocol for Sheep (2015) (Table 3.1). Scoring was conducted by the same observer as in 2021 for the majority of the 2022 trial. Ewe welfare assessments consisted of a BCS by palpation, a visually assessed dag score and a mastitis score by palpation, as well as a visual binary assessment (0 – good condition/1-problem recorded) of the condition of their fleece, breathing, dentition and any injuries. For lambs, I recorded a dag score, and a binary fleece, breathing and injury score. Visual ID numbers were re-painted monthly to allow for continuous clear identification in the field. A faecal sample ranging from 1 to 5 grams was collected per rectum and milk samples of 1 to 3 ml were taken from both halves of ewes' udders for somatic cell counts (SCC). If too little or no milk was extracted, the udder half was marked “empty.” Teats were disinfected using disinfectant wipes soaked in 70% ethanol. The first drops of milk were discarded then samples were collected in sterile 15 ml plastic vials (Vacutainer™, Thermo Fisher Scientific, Waltham, USA). Vials were labelled with ewe ID and stored in a sealed plastic bag labelled with the date, which was transported to the laboratory within two hours. In the laboratory they were stored in a refrigerator at 4°C until processing which occurred within 6 hours. For digital somatic cell counting, a small portion of the milk sample was poured into the vial’s cap, from which disposable cassettes could be filled with 60µL of milk and entered into a DeLaval cell counter (Tetra Laval, Tumba, Sweden). When the machine reported an error after reading the sample, the process was repeated once and if the error persisted, the sample was marked as “empty.”

On a weekly basis, an in-field welfare assessment of ewes and lambs was conducted. A single observer entered the paddock and allowed the sheep to return to their normal behaviour if disturbed for up to 5 minutes. The following measures were recorded in Excel using a tablet (Galaxy Tab A, Samsung, Suwon, South Korea) based on visual observation from a distance, using binoculars when necessary: dag score, fleece score, ewe-lamb distance before weaning and lameness score for ewes and lambs. The dag, fleece and lameness scores were measured using the in-field welfare assessment guide (Table 3.1) created by TechCare and based on the AWIN (2015) scores for field assessment (rather than at handling) to ensure harmonisation across all TechCare trials, as above.

Table 3.1. Welfare assessment scoring scales from (TechCare, 2023) used in the 2022 field trial during scoring at fortnightly handling and weekly in-field assessments.

	Handled score	**In-field score**
Ewe Body Condition Score	**0** – Emaciated (<1 on the Russell BCS range [(Russel et al., 1969)])	**N/A**
	1 – Thin (1 ≤ x ≤ 2 on the Russell BCS range)	
	2- Good (2 < x < 4 on the Russell BCS range)	
	3 - Fat (> 4 on the Russell BCS range)	
Dag score	**0** - No faecal soiling, the wool around the breech area and under the tail is clean	**0** - No faecal soiling, the wool around the breech area and under the tail is clean
	1 - A small quantity of faecal matter can be seen in the wool around the tail	
	2 - Some soiling around the anus and dags in this area only	
	3 - Soiling and dags extending beyond the anus to the tail and upper part of the legs	**1**- Soiling and dags extending beyond the anus to the tail and upper part of the legs
	4 - Wide area of soiling with dags extending down the legs at least as far as the hocks	**2**- Wide area of soiling with dags extending down the legs at least as far as the hocks
Mastitis score	**0** - Normal udder – udder is soft and pliable, no redness or hardness, normal secretions (AWIN 1st level)	**N/A**
	1 - One small fibrotic lump or area of hardness can be felt in the mammary tissue, normal secretion	
	2 - More than 1 lump is present, or areas of hardness on one side of the udder, or small lesion (<10 cm at widest part); milk can be normal or purulent (AWIN 2nd level)	
	3 - Extensive swelling of the udder, lumps or hardness on both sides or larger lump on one side, or lesions >10 cm at widest part. May be absessed or ruptured. (AWIN 3rd level)	
	4 - Peracute mastitis: Complete udder involvement with severe inflammation, secretions range from serum-like to purulent, Mammary	

	lymph nodes enlarged, elevated body temperature.	
	Handled score	**In-field score**
Fleece score	**0** - Sufficient and even fleece cover for breed/time of year; no sign of wool pulls or loss	0- Sufficient and even fleece cover for breed/time of year; no sign of wool pulls or loss
	1 - Loose fleece and shed areas or bald patches, trailing fleece may be present	1- Loose fleece and shed areas or bald patches, trailing fleece may be present
Breathing score	**0** – no laboured breathing or wheezing noises	**N/A**
	1 – any signs of laboured breathing such as wheezing noises	
Injury score	**0** – no injuries recorded	**N/A**
	1 – any injury recorded	
Lameness score	**N/A**	**0** - Movement is smooth, weight is borne equally on all 4 feet with no shortening of stride. Some minor head nodding is allowed if the animal is walking on an uneven surface (field observations).
		1 - Clear shortening of the stride with obvious head nodding or flicking as the affected limb touches the ground
		2 - Very obvious head nodding and not weight-bearing on the affected limb whilst moving, or lame on more than one limb. Foot may be held up whilst standing (hindlimb lameness) or may be seen grazing on knees (forelimb lameness) in field assessment.
		3 - Recumbent or reluctant to stand or move. In field assessments the sheep may not be able to stand or unable to move away from approach. The sheep should not be forced to stand if clearly recumbent.

3.2.4.2 Behavioural observations

i. Data collected in 2021

Observations were carried out by a single observer who was blind to treatment group using scan sampling to record common, maintenance behaviours and behaviour sampling to capture rare, short-duration behaviours such as playing and sucking events. Four times a week, the sheep were observed starting at 7:00am to avoid disturbances such as farm management activities. The observer entered the paddock on foot and allowed up to 5 minutes for normal sheep behaviour to resume. Two scan samples were conducted on all individuals by walking the entire paddock and using binoculars if necessary to avoid disturbing the sheep. Sampling began with the sheep closest to the observer and continued until every individual was observed. The ethogram in Table 3.2 was used and a 30-minute interval was left between the two scan samples. Immediately following scan sampling, a lameness score (AWIN, 2015) was assigned to every individual. The ewe-lamb distance for each lamb was recorded using the distance between two fence posts (2 metres) as a reference. Scan sample results were entered into Microsoft Excel on a tablet during sampling (Galaxy Tab A, Samsung, Suwon, South Korea). After the first scan sample, a 30-minute behaviour sample was taken using the ethogram in Table 3.3. The behaviour sample was a focal sample carried out on the entire flock, conducted from an observation seat 3 metres off the ground, allowing an unobstructed view of all animals. All occurrences of behaviours described in Table 3.3 were recorded, along with the individuals who expressed them and their duration. Behaviour samples were dictated into a smart phone voice recording app (Apple, Cupertino, USA) and transcribed into Microsoft Excel the same day. Following the behaviour sample, the second scan sample was carried out using the protocol described above.

Table 3.2. Ethogram for 2021 scan sampling of ewe and lamb maintenance behaviours.

Behaviour	**Description**
Grazing/Drinking	Chewing or obtaining grass or foliage, or water from trough, with head down below the shoulders within 10cm of the ground while lifting one or more feet off the ground and moving forward or with four feet not leaving the ground.
Locomotion	Moving feet, leading to motion in any direction for more than 2 seconds.
Lying	Animal's body is touching the ground from shoulder to back end, neck and head touching the ground or upright.
Standing	Remaining still in a posture where head is raised above the level of the back, up on all four legs.
Scratching	Rubbing body or head against fencing, tree or water trough.
Ruminating	Resting with whole body on ground off all four feet, head up above shoulder level, regurgitation or chewing by moving bottom jaw for more than 5 seconds

Table 3.3. Ethogram for 2021 behaviour sampling of ewe and lamb social behaviours.

Behaviour	**Description**
Locomotor Play	Moving rapidly in any direction for more than 2 seconds with no obvious destination to reach, jumping or pivoting for no obvious reason
Social play	Putting head down and running to butt heads with another sheep, or mounting another sheep
Sucking	Lamb's head within 5 cm of ewe's udder for more than 2 seconds

ii. Data collected in 2022

The ethogram from 2021 was adapted to describe the sheep's behaviour and posture in more detail, for example grazing was recorded as grazing active or grazing inactive depending on whether locomotion occurred at the same time as grazing. This change was made after reflection on the behavioural motivations that could be most affected by disease, e.g. a ewe may still be motivated to eat when lame but her will to walk would be decreased, resulting in grazing on the spot rather than walking and grazing at the same time. Four scan samples were collected four times a week at 2-hour intervals using the ethogram in Table 3.4. They were collected according to the same procedure described above for 2021. The time at which each individual animal was observed was recorded in an hh:mm:ss format. No behaviour samples were recorded in 2022.

Table 3.4. Scan sample ethogram used in 2022 for ewes and lambs

Behaviour	**Description**
Grazing Active	Chewing or obtaining grass or foliage with head down below the shoulders while lifting one or more feet off the ground and moving forward.
Grazing Inactive	Chewing or obtaining grass or foliage with head down below the shoulders.
Locomotion	Placing feet one in front of the other in a forward motion with head at or above shoulder level
Lying	Resting with whole body on ground off all four feet, absence of other behaviour
Lying ruminating	Resting with whole body on ground off all four feet, head up above shoulder level, regurgitating or chewing by moving bottom jaw for more than 5 seconds
Standing alert	Remaining still in a posture where head is raised above the level of the back, weight is placed on all four legs, absence of other behaviour.
Standing ruminating	Remaining still in a posture where head is raised above the level of the back, weight placed on all four legs, regurgitating or chewing by moving bottom jaw for more than 5 seconds
Play	Lamb running with no obvious destination to reach, jumping or pivoting for no obvious reason, butting or mounting another lamb
Social behaviour	Being in active physical contact with another sheep, including nudging, nuzzling, or nosing
Scratching	Standing and rubbing body or head against fencing, tree or water trough or nosing a part of the body repeatedly.
Sucking	Lamb's mouth in contract with or within 10 cm of the ewe's udder for longer than 2 seconds
Unclear	Animal's behaviour is concealed by a visual barrier e.g. bush or another ewe/lamb.

3.2.4.3 Qualitative Behaviour Assessment (QBA)

i. Data collected in 2021

QBA was carried out on the lambs by the same observer once a week at the same time every week. The observer let sufficient time pass for the animals to settle after entering the paddock before initiating the QBA. Once the animals had resumed their ongoing behaviour, each lamb was observed for 1 minute. The same list of terms used for QBA in Chapter 2 (AWIN, 2015) (Table 3.5) was applied to score the animals' demeanour using the SRUC QBA App running on a tablet (Xperia S, Sony Europe Ltd., Weybridge, UK).

ii. Data collected in 2022

QBA was carried out weekly in the same way as in 2021, however both ewes and lambs were observed. The list of terms was shortened compared to 2021 (Table 3.5) by eliminating terms that were highly correlated with other terms in the 2021 analysis. Scores were recorded on an Android smartphone rather than a tablet using the same app. Due to differences in design across the apps on different devices, the scoring scales were different across years, even when using the same terms. For this reason, the QBA results from 2021 and 2022 will be presented separately in this study.

Table 3.5. Qualitative Behaviour Assessment (QBA) terms used to describe sheep's behaviour in 2021 and 2022 and their definitions.

Term	**Definition**	**Used 2021**	**Used 2022**
Alert	Observant and vigilant	Yes	Yes
Active	Animal is physically active. Engaged in task e.g. grazing, walking, or fighting.	Yes	Yes
Relaxed	At ease, free from anxiety, agitation or tension. The animal appears to be unthreatened.	Yes	Yes
Fearful	Attention is focussed on one specific object/being which is either a real or perceived threat. Animal may also be fleeing.	Yes	Yes
Content	Satisfied and at peace. The animal's needs are met, or the animal is successfully working towards their completion.	Yes	Yes
Agitated	Excessive cognitive and/or motor activity due to tension or anxiety. The animal is uneasy and if moving their actions are twitchy.	Yes	Yes
Sociable	Seeking and interacting with other sheep. The sheep appears to be enjoying/taking comfort from their contact. The sheep is choosing to be part of a flock and not fully isolate themselves	Yes	Yes
Aggressive	Hostile and tense. Attacking/ready to attack, usually unprovoked or to compete for resource	Yes	Yes
Vigorous	The animal is carrying out task in an energetic or forceful way. If stationary or moving slowly the animal expresses an inner strength and energy. May imply good physical health.	Yes	Yes
Subdued	Submissive and docile. Often removed from social group and self absorbed.	Yes	Yes
Physically uncomfortable	Giving impression of pain or other physical discomfort through posture/movement	Yes	Yes
Defensive	Ready to potentially defend herself or lamb from harm/perceived threat	Yes	No
Calm	Placid and sedate. If physically active the animal's movements are smooth and unhurried.	Yes	Yes
Frustrated	Dissatisfied. Unable to fulfil satisfaction and achieve goal.	Yes	Yes
Apathetic	Unresponsive and dull.	Yes	No
Wary	Shy, cautious, apprehensive and possibly distrustful.	Yes	Yes
Tense	Uneasy and/or on-edge. Posture may show physical tension.	Yes	No
Bright	Alert, lively and aware of environment.	Yes	Yes
Inquisitive	Curious, interested and intrigued by the environment or other animals.	Yes	Yes
Listless	Lack of vigour and energy. Animal appears lacklustre	Yes	Yes
Assertive	Displaying confidence or determination	Yes	No

3.2.5 Data Analysis

3.2.5.1 Combining years for analysis

Datasets were processed and stored in Microsoft Excel. All statistical analysis was conducted in R version 4.2.3 (R Core Team, 2023) via R Studio (version 3.0). Ewe and lamb data were analysed separately. Data from 2021 and 2022 were combined for analysis in the following ways.

The 2021 and 2022 scan sampling ethograms (Tables 3.2 and 3.4) were combined to allow for behavioural analysis using data from both years (Table 3.6). Mastitis score data from 2021 were placed on the 2022 scale for analysis. Where measurements were only taken in one year (e.g. SCC in 2022 only), only results from that year are presented.

Table 3.6. Behaviours from 2021 and 2022 ethograms and how they were combined to form a new set of "Combined analysis behaviour categories."

Combined analysis behaviour categories	**Behaviours included from 2022 ethogram**	**Behaviours included from 2021 ethogram**
Grazing	Grazing Active, Grazing Inactive	Grazing/Drinking
Locomotion	Locomotion	Locomotion
Lying	Lying, Lying ruminating	Lying
Standing inactive	Standing alert	Standing
Play	Play	Social play, Locomotor Play
Social behaviour	Social behaviour	Social behaviour
Scratching	Scratching	Scratching
Ruminating	Lying ruminating, Standing ruminating	Ruminating
Sucking	Sucking	Sucking
Unclear	Unclear	Unclear

3.2.5.2 Behavioural Data Analysis

To determine the relationships between the binary behaviours (presence / absence (0,1)) performed during scan sampling and the welfare indicators, Generalised Linear Mixed Models (GLMM) [glmmTMB package (Brooks et al., 2017)] were performed. Behaviours performed in less than 5% of total observations were not analysed due to the lack of variation and low incidence. A negative binomial distribution with a quadratic parameterization (nbinom2) link function was applied. Fixed effects included lameness (0, 1, 2, 3, 4), presence of a collar (Y,N), scan number (1, 2, 3, 4), while strongyle FEC (eggs/gram) and *Nematodirus* FEC (eggs/gram) were included as covariates. Interaction terms were lameness*strongyle, lameness**Nematodirus* and *Nematodirus**strongyle to investigate any interactions between welfare conditions. Family ID (a number assigned to each replicate) with day of experiment (DOE) nested within it was included as a random effect, except when this nesting prevented models from running, in which case DOE was included as a covariate. Dag, fleece, breathing and injury scores in ewes and lambs did not contain enough variation to be included in the analysis. Mastitis, SCC, BCS, dentition score, strongyle and *Nematodirus* variables as well as interaction terms were not included in ewe behaviour models because they did not contain enough variation and prevented models from running.

Analysis of behaviour samples examined the relationship between play and sucking behaviours with welfare indicators. It used GLMMs with *glmmTMB* (Brooks et al., 2017) to study total durations and bout counts of play and sucking for each sample. Social play and locomotor play were combined into a single variable called Play. The family link function was set to either negative binomial distribution with a quadratic parameterization (nbinom2), Gaussian or Poisson distribution, dependent on model fit and overdispersion parameters (Hardin & Hilbe, 2007). Strongyle FEC, N*ematodirus* FEC and DOE acted as covariates, and technology treatment group (Y,N) and lameness (0,1,2,3) were the fixed effects. To account for variation across individuals, lamb ID acted as a random effect.

Ewe-lamb distance models were built separately for ewes and lambs, meaning they featured the same explanatory variables as the binary behavioural models described above, but were run once with lamb data populating the welfare variables and a second time with ewe data. A lack of variation in the lamb data prevented these models from running unless ewe-lamb distance was turned into a binary measure, where 0 represented a distance of less than one metre, and 1 represented one metre or more. Using the *glmmTMB* package (Brooks et al., 2017), binomial GLMMs were therefore built to examine how ewe-lamb distance is affected by lamb parameters, whereas a negative binomial distribution with a quadratic parameterization (nbinom2) link function was used to question the effects of ewe parameters.

For all GLMMs, model fit was confirmed using the *DHARMa* package (Hartig, 2022), and the residuals of all models were in accordance with uniformity assumptions (Hartig, 2022). The ANOVA function in the *car* package (Fox & Weisberg, 2018) was used to determine the significance of explanatory variables based on a $p<0.05$ threshold and to examine differences between fixed effects and interactions. Pairwise comparisons of estimated marginal means (i.e. adjusted or least-squares means) and associated standard errors were derived with the *emmeans* function from the *emmeans* package (Lenth, 2023) with mode set to “response” to obtain estimates in the response scale, with Tukey adjustment of p-values accounting for multiplicity. *Emmeans* (Lenth, 2023) was also used to examine linear trends between fixed effects and covariates. Graphical representations of results were produced using *ggplot2* (Wickham, 2016) with corrected pairwise comparisons with standard error (SE) and 95% confidence intervals (CIs) reported.

3.2.5.3 Qualitative Behaviour Assessment

Principal Component Analysis (PCA) (Wold et al., 1987) was used to obtain the distribution of lamb and ewe affective states, and GLMMs were used to analyse the loadings on the principal components across time. In the 2022 dataset, where data was collected on ewes and lambs, these two groups were analysed separately. A PCA was run on the scores for all descriptive terms (21 total in 2021 and 17 in 2022) across observations using the *prcomp* function from the *stats* package (R Core Team, 2023). A scree plot was produced using the package *factoextra* (Kassambra & Mundt, 2020) and the dimensions that accounted for the highest levels of variance were retained for graphical representation and modelling using the Elbow method (Joshi & Nalwade, 2013). The base R function *print* was applied to the resulting PCA to produce a covariance matrix of terms and the PCA dimensions. This allowed for interpretation of each dimension. Using the package *factoextra* (Kassambra & Mundt, 2020), the dimensions of interest were graphically populated with individuals. *factoextra* (Kassambra & Mundt, 2020) was also used to extract the coordinates of each observation along the first two dimensions in 2022 and three dimensions in 2021.

Due to server issues, QBA data from Phase 2-2021 were lost, so only data from Phase 1-2021 were used in analysis. To test if FEC and time had any effect on lambs’ loadings along the three dimensions, Gaussian GLMMs were built. (Y+10) was used to account for negative values in the response variable without disrupting variance and squaring the response variable was necessary when lamb loadings along PC2 were modelled. The results reported below have been back transformed. Fixed effects included technology treatment group (Y,N), and week of experiment (1,2,3,4), while strongyle and *Nematodirus* FEC were covariates. An interaction between week and technology was included and Animal ID acted as the random effect. A Kendall's coefficient of concordance was then calculated using the *KendallW* function from the *DescTools* package (Signorell, 2023) to compare the ranking of individuals along dimensions by their loadings. This allowed us to track individuals through time by examining if the individual sheep's ranks along dimensions were consistent across the four weeks of observation.

To compare the affective states of ewes and lambs before and after a welfare challenge such as weaning in 2022, Gaussian GLMMs were built using QBA data from one week prior and one week following weaning. (Y+5) or (Y+10) was used to account for negative values in the response variable without disrupting variance and squaring the response variable was necessary when lamb loadings along PC2 were modelled. The results reported below have been back transformed. Fixed effects included technology treatment group (Y,N) and weaning status (whether the observation was recorded before or after weaning [0,1]), and the interaction between these two terms. Covariates were strongyle and *Nematodirus* FEC. Animal ID was included as the random effect. A Kendall's coefficient of concordance was then calculated using the *KendallW* function from the *DescTools* package (Signorell, 2023) to compare the ranking of individuals along both dimensions. This allowed us to understand if the individual sheep's ranks along both dimensions were consistent before and after weaning.

3.3 Results

3.3.1 2021 Treatment groups and faecal egg counts (FEC)

When analysing data from the entire 2021 study, there was a significant difference in strongyle FEC between HP and LP lambs ($p<0.001$) (Figure 3.1c). However, there was no significant difference in *Nematodirus* FEC between HP and LP lambs ($p=0.813$) (Figure 3.1a). There were no differences in *Nematodirus* ($p=0.735$) or strongyle ($p=0.410$) FEC between HP ewes and LP ewes (Figure 3.1b and 3.1d).

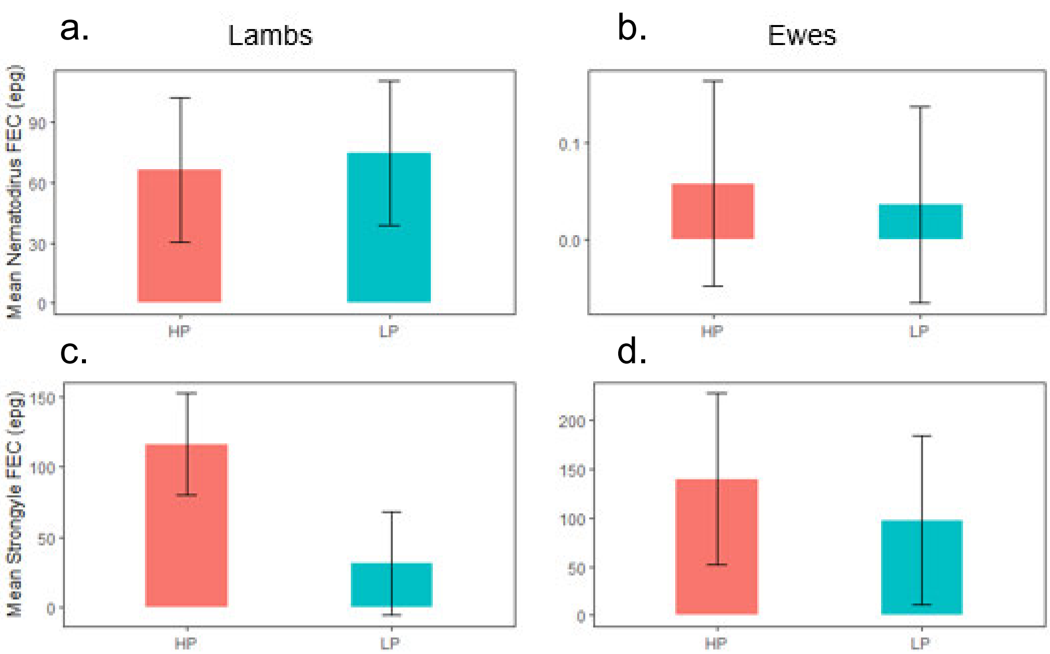

Figure 3.1. Comparison of the Nematodirus *faecal egg counts of HP and LP lambs (a) and ewes (b) and strongyle faecal egg counts of HP and LP lambs (c) and ewes (d) in 2021.*

3.3.2 Welfare Indicators

Ewe FEC remained low for strongyles and extremely low for *Nematodirus*, as expected due to their immunity developed over years of exposure (Figure 3.2 and 3.3). Ewe dag score was more variable in 2022 than 2021 (Figure 3.2 and 3.3). Mastitis scores above 0 were recorded at every sampling event, with more severe cases recorded in 2022 than 2021 (Figures 3.2 and 3.3). Somatic cell counts in 2022 were mostly indicative of a lack of clinical mastitis cases (defined as SCC > 1000×10^4 cells/mL), but a handful of individuals with high counts brought up the means (Figure 3.3). BCS records show the highest proportion of thin ewes early in the grazing season in both years (Figures 3.2 and 3.3).

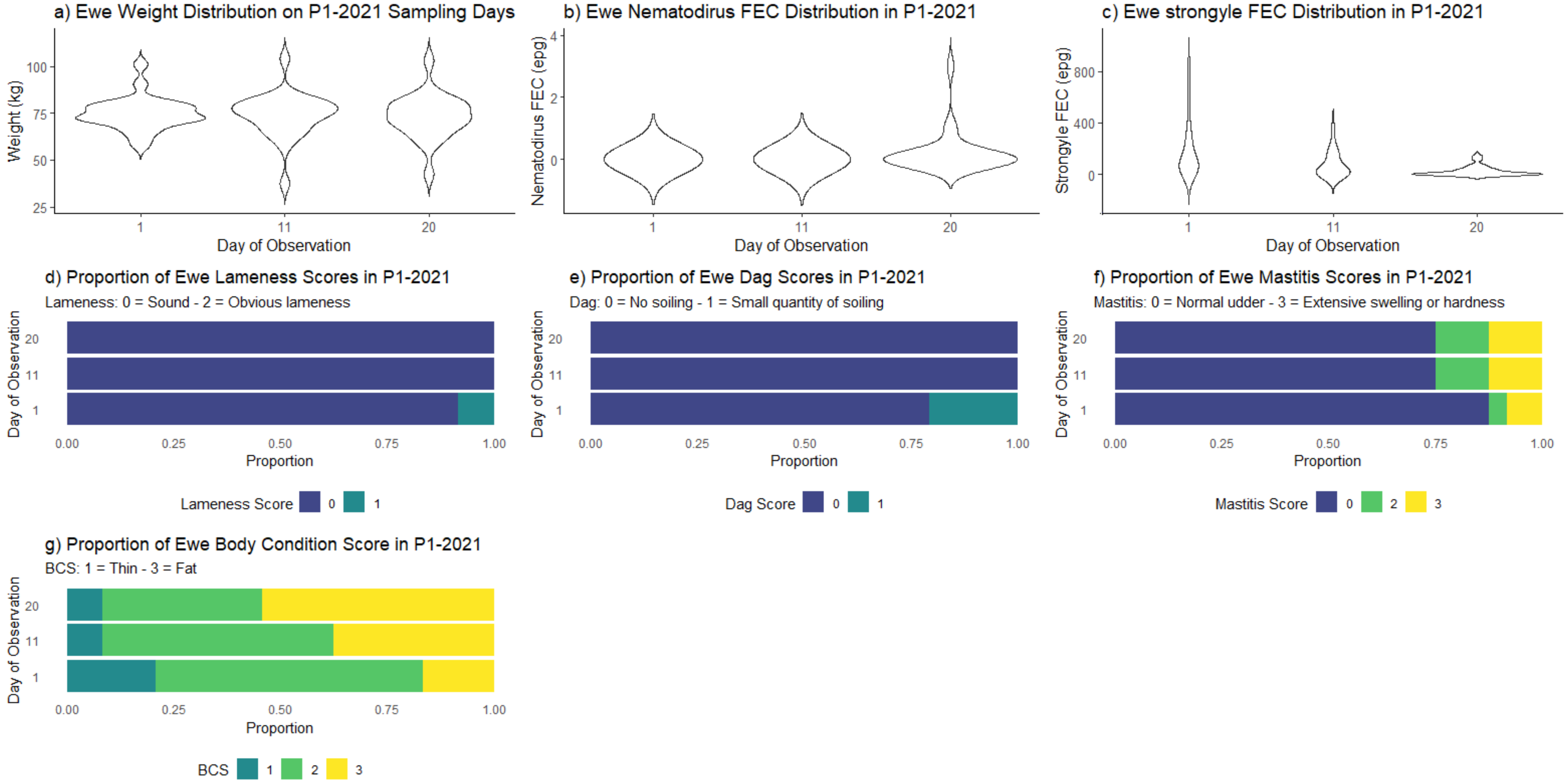

Figure 3.2. Descriptive statistics of ewe welfare indicators in 2021: weight (a), Nematodirus FEC (b), Strongyle FEC (c), lameness (d), dag score (e), mastitis (f) and body condition score (g).

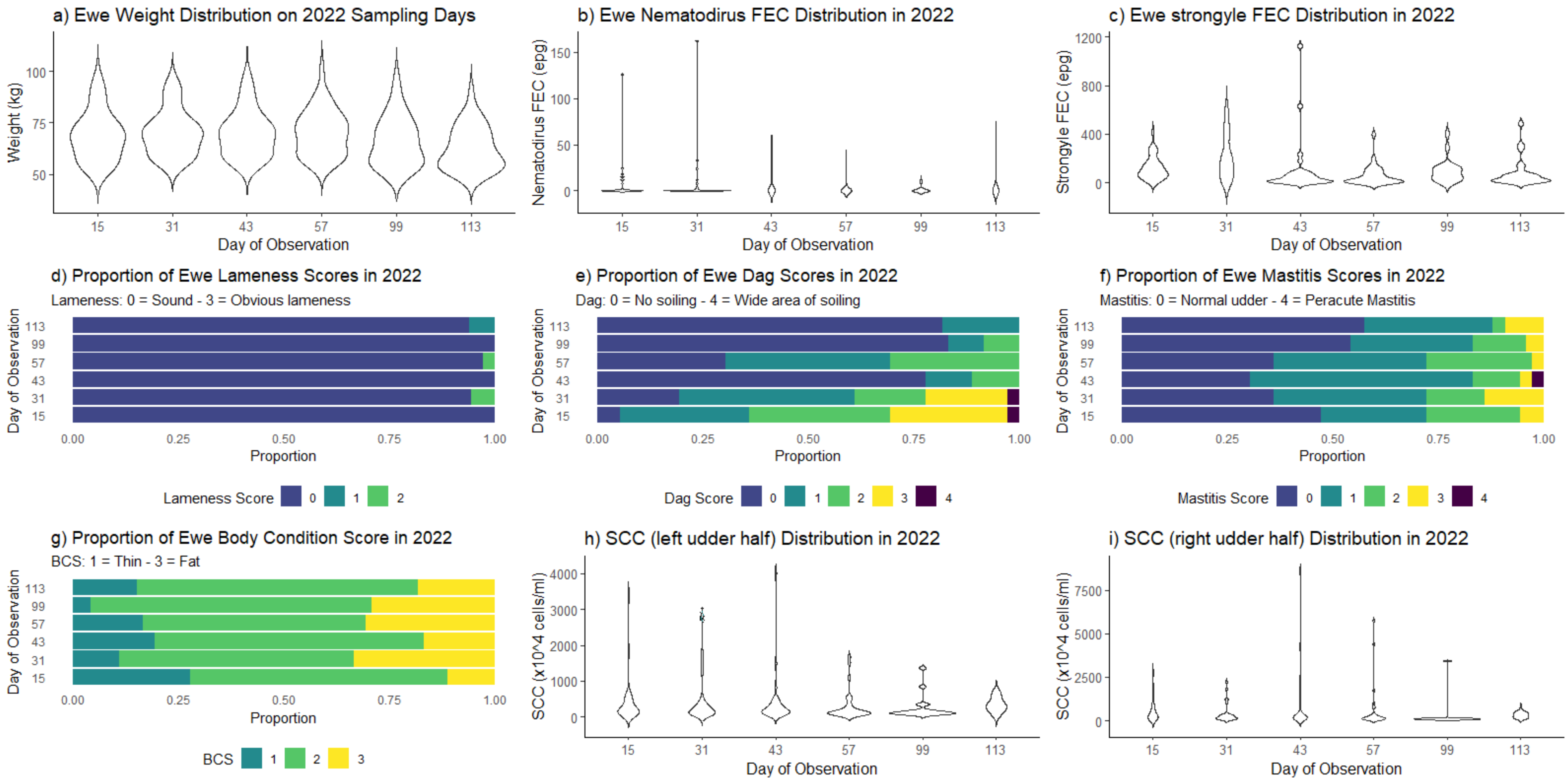

Figure 3.3. Descriptive statistics of ewe welfare indicators in 2022: weight (a), Nematodirus FEC (b), Strongyle FEC (c), lameness (d), dag score (e), mastitis (f) and body condition score (g), somatic cell count (SCC) in the left udder half (h) and the right udder half (i).

Lamb weight increased then stabilised over time, as expected (Figures 3.4 and 3.5). Lower weights were recorded in 2022 because sampling began two weeks after birth compared to eight weeks after birth in 2021. In both years, higher values of *Nematodirus* FEC occurred at the beginning of observation and then decreased, while higher strongyle FEC occurrences were recorded later in the season (Figures 3.4 and 3.5). However, some lambs had high strongyle FEC (up to 750 epg) as early as day 58 in 2022, which was the same day the highest *Nematodirus* FEC values were recorded (Figure 3.5). Lameness was recorded in 1.8% of observations over both years, with more mildly lame lambs in 2021 compared to a small percentage of more severely lame lambs in 2022 (Figures 3.4 and 3.5). Dag scores were highest later in the season in both years (Figures 3.4 and 3.5). Overall, marginally less than half of observations were given a dag score of 1 or above (). Breathing, fleece and injury scores in 2022 remained low (Figure 3.5).

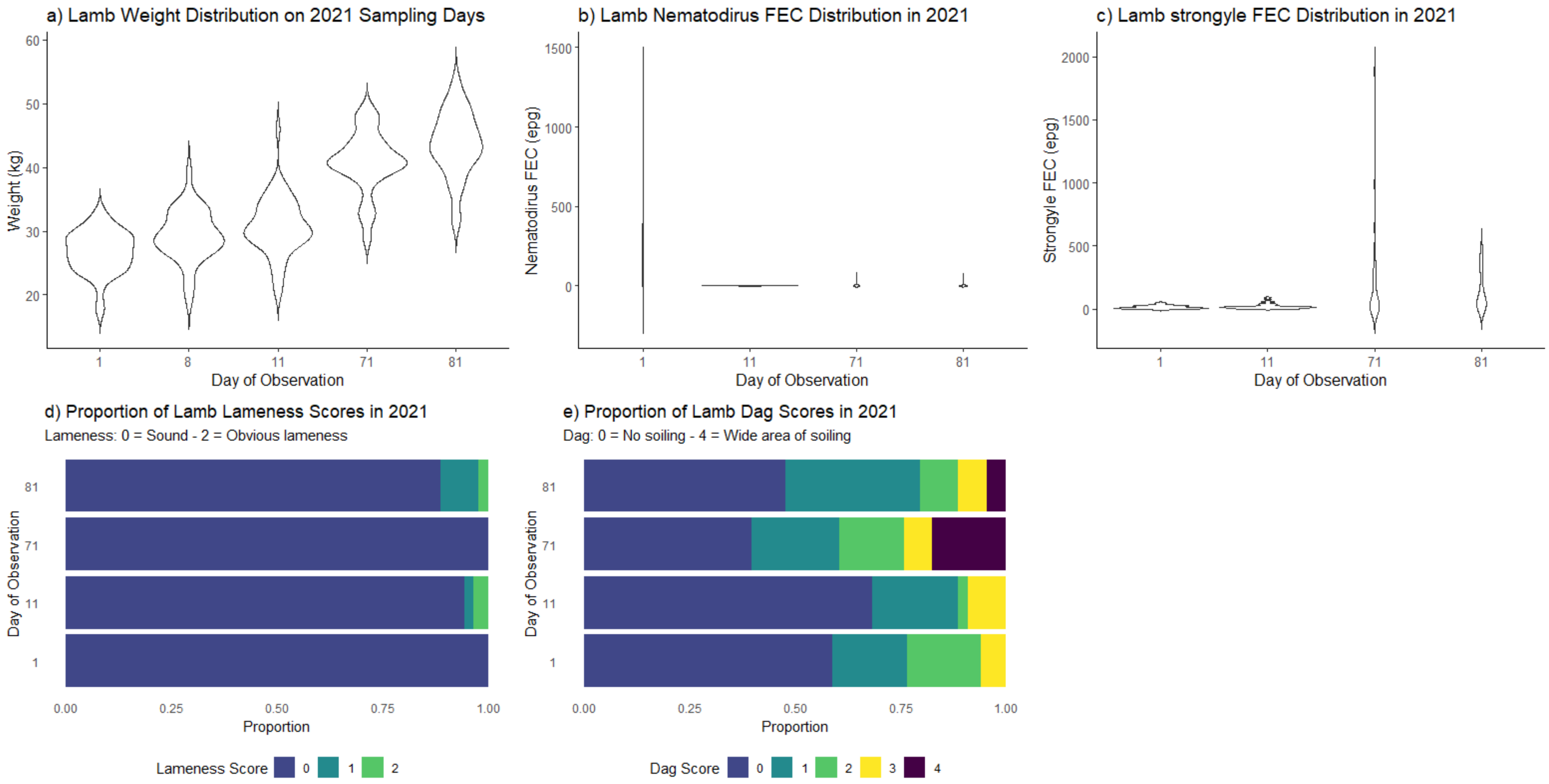

Figure 3.4. Descriptive statistics of lamb welfare indicators in 2021: weight (a), Nematodirus FEC (b), Strongyle FEC (c), lameness (d), dag score (e).

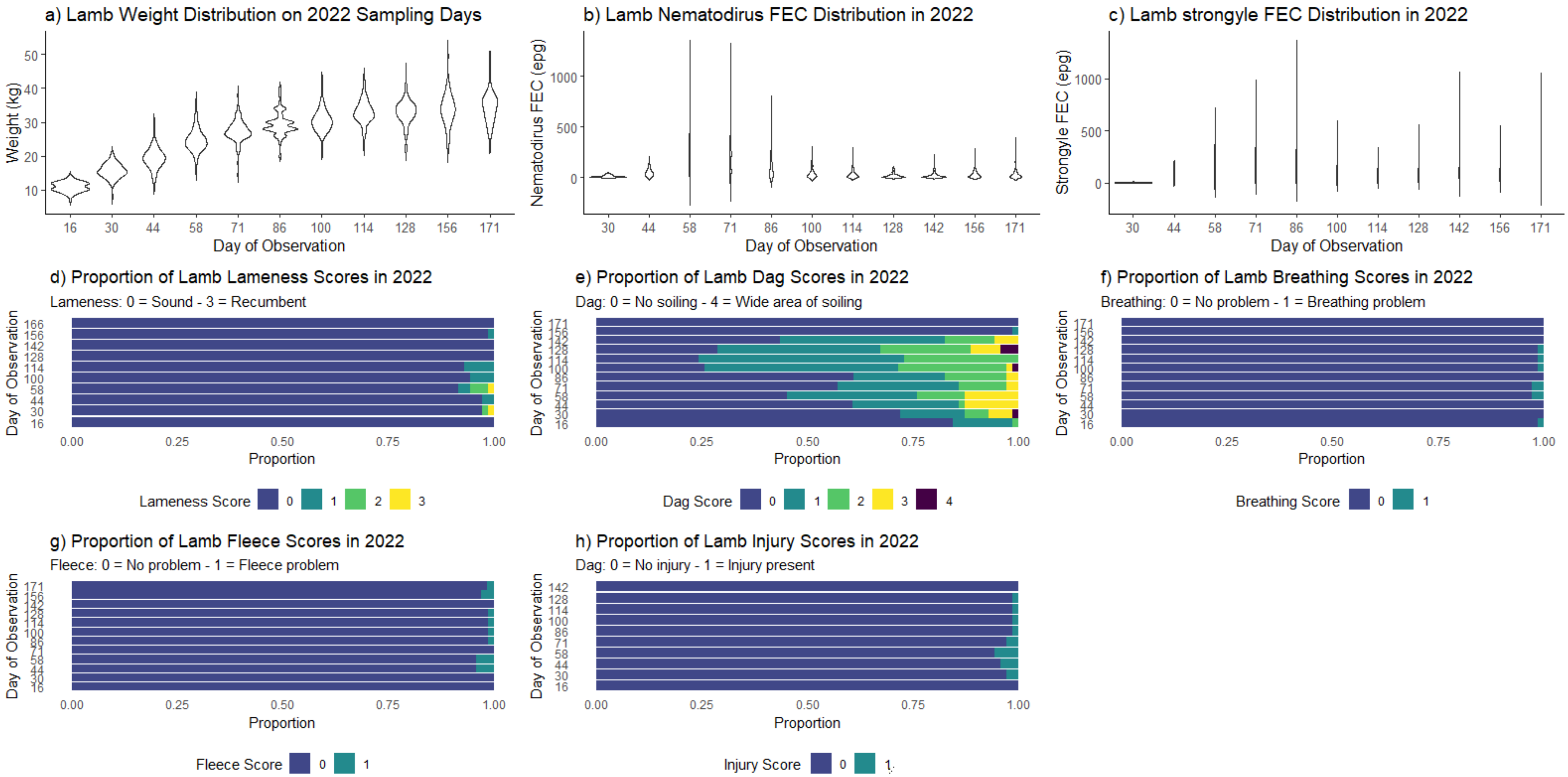

Figure 3.5. Descriptive statistics of lamb welfare indicators in 2022: weight (a), Nematodirus FEC (b), Strongyle FEC (c), lameness (d), dag score (e), breathing score (f), fleece score (g), injury score (h).

3.3.3 Behaviour

3.3.3.1 Scan Samples

i. Lambs

The most common behaviour recorded in lambs across both years was grazing, and the least common was play in 2021 and social behaviour in 2022 (Figure 3.6). Locomotion was negatively associated with day of experiment (est=-0.006, SE=0.0008, z=-8.027, $p<0.001$), meaning lambs performed fewer instances of locomotion as time went on (Figure 3.6). Similarly, lying was negatively associated with day of experiment (est= -0.024, SE= 0.0009, z= -26.725, $p<0.001$) (Figure 3.6). There was a higher probability of lambs ruminating in 2022 (prob=0.09, SE=0.05) than in 2021 (prob=0.04, SE=0.02) ($p<0.001$), meaning lambs were recorded ruminating in 2022 significantly more often than in 2021 (Figure 3.6). Similarly, there was a higher probability of lambs lying in 2022 (prob=0.068, SE=0.022) than in 2021 (prob=0.025, SE=0.009) ($p<0.001$) (Figure 3.6). However, lambs were recorded standing more often in 2021 (prob=0.822, SE=0.395) than in 2022 (prob=0.639, SE=0.623) ($p<0.001$) (Figure 3.6). The frequency of other behaviours did not significantly differ across years.

ii. Ewes

In ewes, grazing was the most recorded behaviour in both years, while scratching and social behaviour were the rarest in 2021 and sucking (meaning lambs sucking at the ewe's udder with the absence of any other behaviour from the ewe) was least often recorded in 2022 (Figure 3.7). Ewes were recorded grazing more often in 2022 (prob=0.423, SE=0.041) than in 2021 (prob=0.320, SE=0.041)($p<0.001$) (Figure 3.7). Similarly, locomotion was recorded more often in ewes in 2022 (prob=0.087, SE=0.021) than in 2021 (prob=0.044, SE=0.013) ($p<0.001$) (Figure 3.7). Lying was recorded more frequently in 2021 (prob=0.112, SE=0.040) than in 2022 (prob=0.053, SE=0.019)($p<0.001$) (Figure 3.7). Finally, ewes in 2021 (prob=0.311, SE=0.041) were recorded as ruminating more often than ewes in 2022 (prob=0.253, SE=0.031) ($p=0.012$) (Figure 3.7). The frequency of other behaviours in ewes did not differ significantly across years. These differences likely arose due to the higher number of samples taken in 2022.

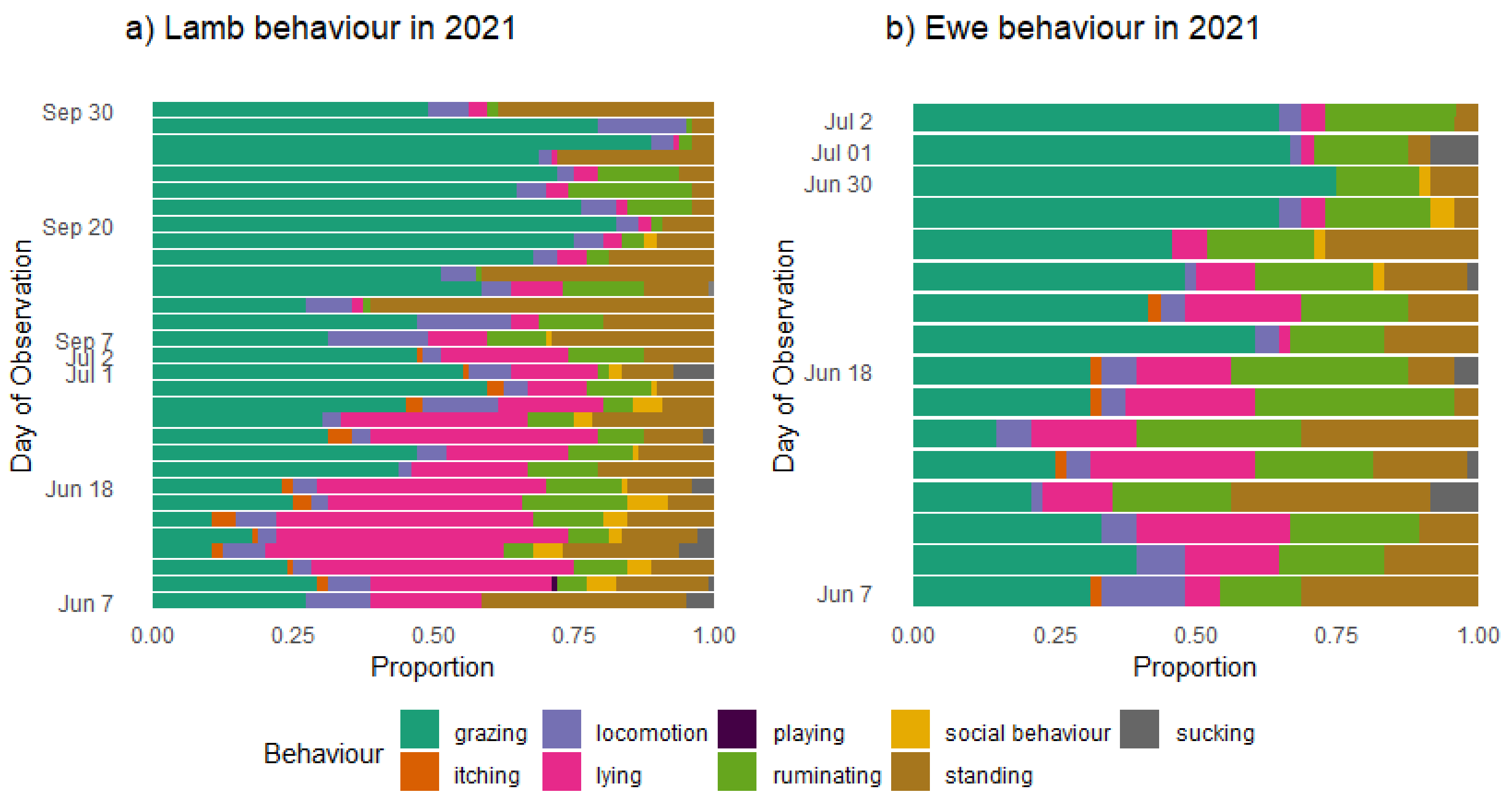

Figure 3.6 Proportions of lamb (a) and ewe (b) behaviours recorded on every day of observation in 2021.

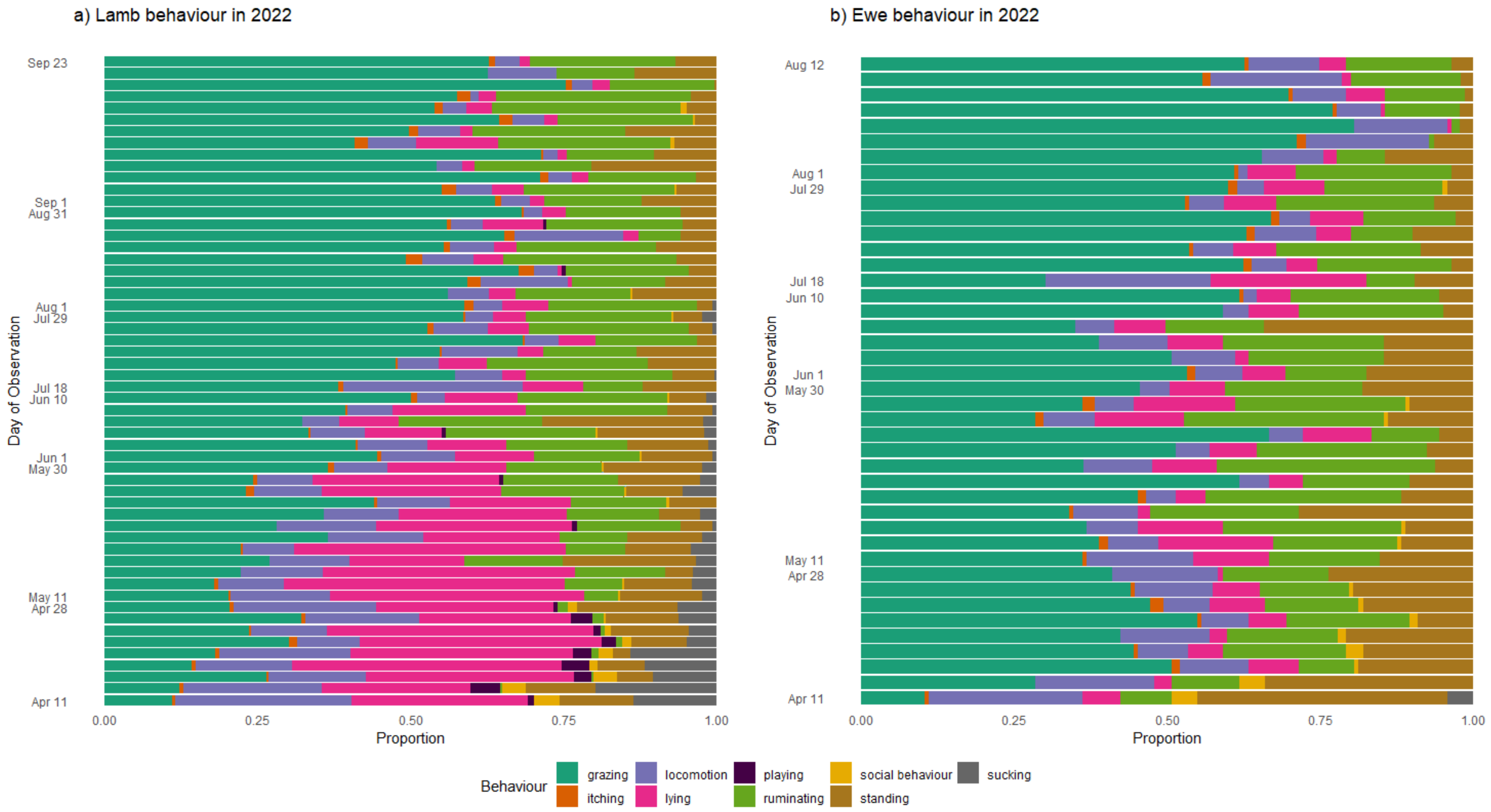

Figure 3.7. Proportions of lamb (a) and ewe (b) behaviours recorded on every day of observation in 2022.

3.3.3.2 Behaviour samples

Over 62 behaviour samples in 2021 of 30 minutes each (a total of 1860 minutes of observations), the lambs performed social play, locomotor play and sucking behaviour 16, 10 and 53 times respectively (Table 3.7). Only 7 out of 48 lambs displayed any play behaviour across all the samples. When social and locomotor play were combined into one variable, play total duration was negatively associated with day of experiment (est= -1.39, SE=0.55, z= -2.55, p=0.011), meaning the amount of time lambs were observed playing decreased as lambs got older. No behaviour samples were collected in 2022.

Table 3.7. Total number of bouts, total duration of bouts in seconds, and average duration of bouts in seconds for social play, locomotor play and sucking in lambs by parasitology treatment group (HP=high parasitism, LP=low parasitism) recorded during behaviour sampling.

		HP	LP	p-value of HP/LP effect
Social Play	Count of events	8	8	0.548
	Total duration of events	121	145	0.753
	Average duration of bouts	13.75	17.85	0.247
Locomotor Play	Count of events	6	4	0.884
	Total duration of events	25	15	0.793
	Average duration of bouts	2.9	3.75	Model does not converge
Sucking	Count of events	31	18	0.967
	Total duration of events	317	207	0.961
	Average duration of bouts	10.81	12.31	0.334

3.3.3.3 Ewe-lamb distance

Ewe-lamb distance was recorded when the lambs were aged 8 to 12 weeks in 2021, but it was recorded from birth until weaning in 2022. The mean distance was significantly higher in 2022 than in 2021 (p<0.001) (Figure 3.8). When analysed in a binomial model (where 0 is ≤1m and 1 is >1m), ewe-lamb distance was positively associated with day of experiment (est=0.003, SE=0.001, z=3.295, p<0.001), meaning it increased as the lambs grew older.

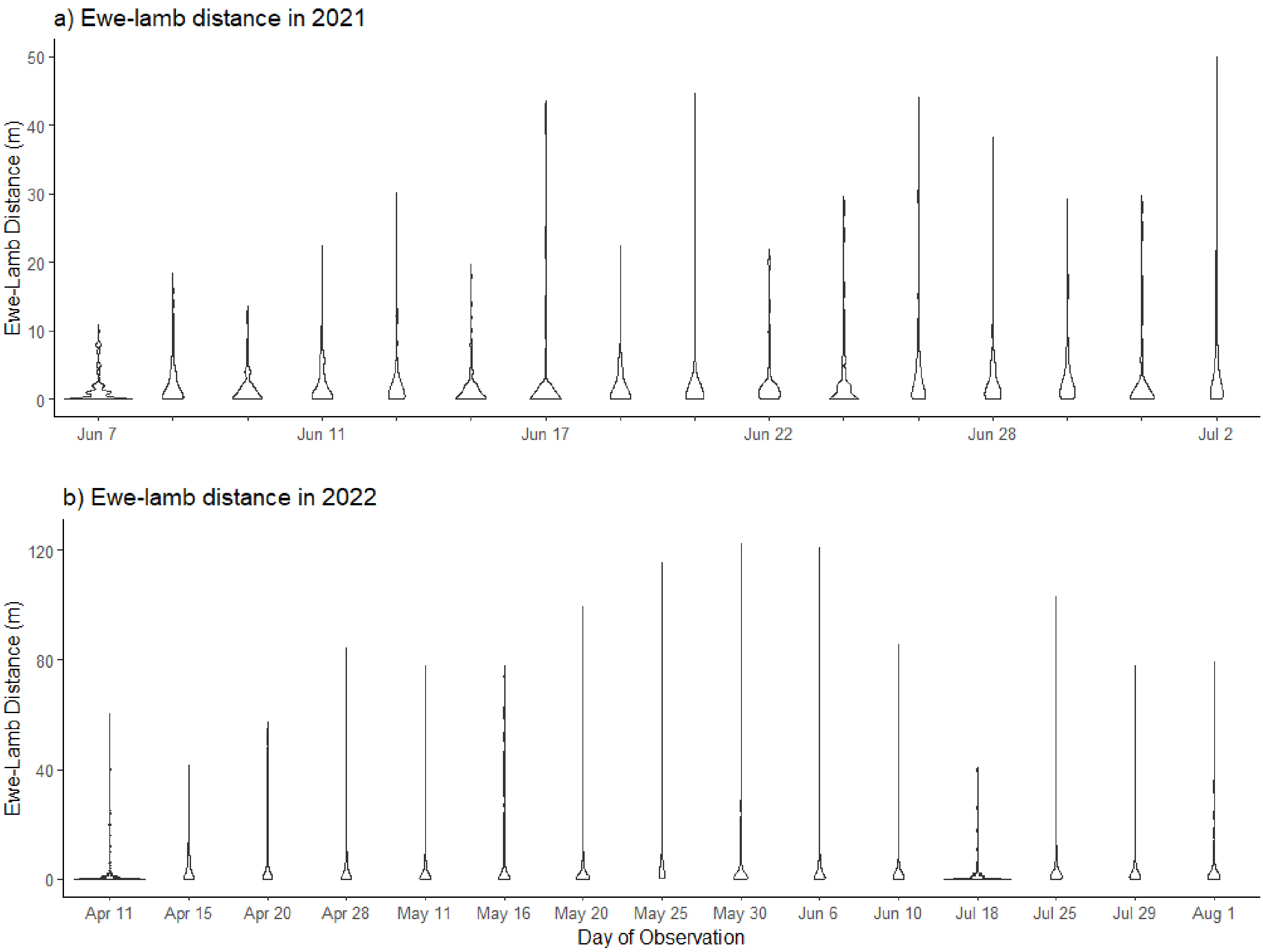

Figure 3.8. Ewe-lamb distance distribution in 2021 (a) and 2022 (b).

3.3.4 Associations between behaviour and welfare

3.3.4.1 Grazing

Lambs with a lameness score of 0 (prob=0.518, SE=0.018) were more likely to be grazing than lambs with a score of 1 (prob=0.290, SE=0.050) (OR=2.640, SE=0.625, 95% CI=1.436-4.850, $p<0.001$) or than lambs scoring 2 (prob=0.206, SE=0.067) (OR=4.140, SE=1.691, 95% CI=1.448-11.820, p=0.003) (Figure 3.9).

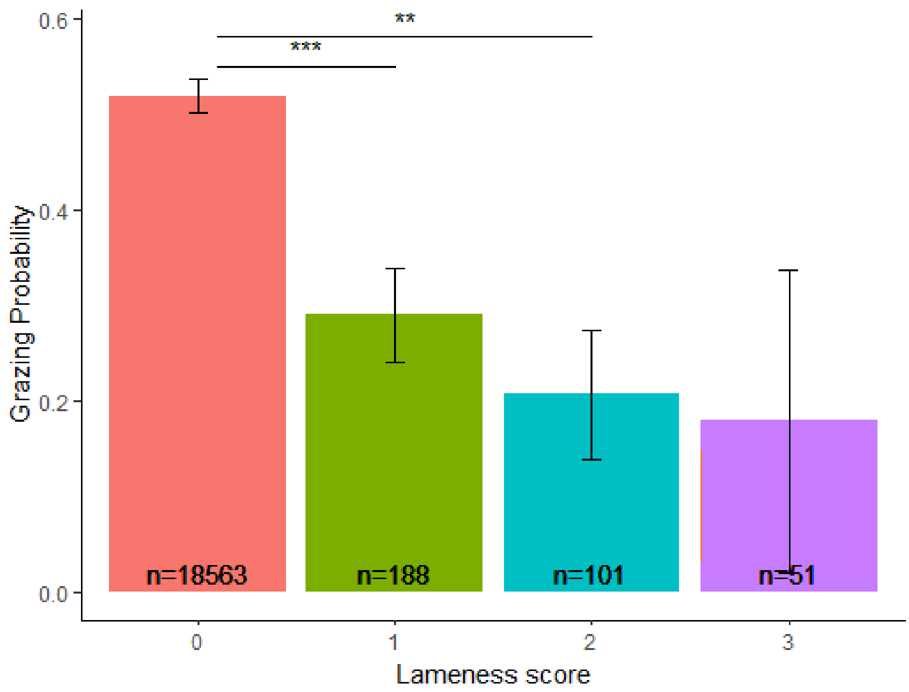

Figure 3.9. Grazing probability of lambs with different lameness scores where n=number of observations in each category, and where 0="Movement is smooth, weight is borne equally on all 4 feet with no shortening of stride", 1="Clear shortening of the stride with obvious head nodding or flicking as the affected limb touches the ground", 2="Very obvious head nodding and not weight-bearing on the affected limb whilst moving, or lame on more than one limb", 3="Recumbent or reluctant to stand or move."

Lamb strongyle FEC was positively associated with grazing (est=0.002, SE=0.0002, z=8.93, p<0.001) while lamb *Nematodirus* FEC was negatively correlated with grazing behaviour (est= -0.001, SE=0.0003, z= -3.31, p<0.001) (Figure 3.10).

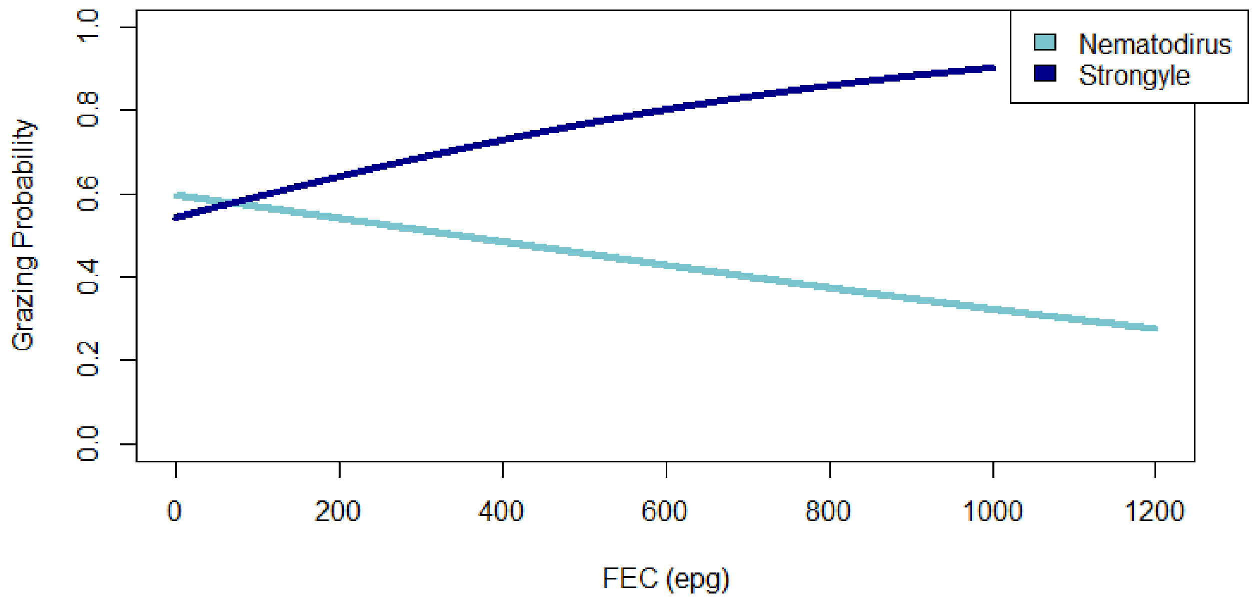

Figure 3.10. Lamb Nematodirus (light blue) and strongyle (dark blue) faecal egg counts (FEC) and their probability of grazing.

3.3.4.2 Locomotion

In lambs, the interaction between lameness and strongyle FEC was significant (est= -0.013, SE=0.005, z= -2.752, p=0.030). Lambs with a lameness score 1 had a negative relationship between strongyle FEC and locomotion, but lambs with a lameness score 2 had the opposite relationship, where their locomotion was positively associated with strongyle FEC (Figure 3.11).

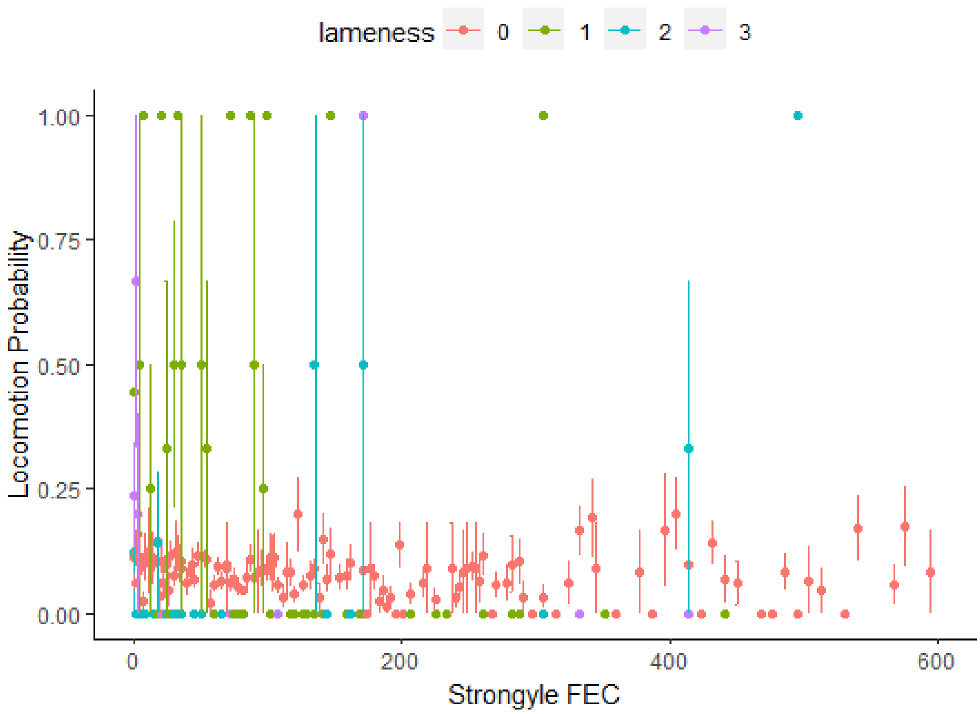

Figure 3.11. Locomotion probability of lambs with different strongyle faecal egg counts (FEC) and lameness scores, where 0="Movement is smooth, weight is borne equally on all 4 feet with no shortening of stride", 1="Clear shortening of the stride with obvious head nodding or flicking as the affected limb touches the ground", 2="Very obvious head nodding and not weight-bearing on the affected limb whilst moving, or lame on more than one limb", 3="Recumbent or reluctant to stand or move."

3.3.4.3 Lying

Lamb *Nematodirus* FEC had a very small but significant negative relationship with lying behaviour (est= -0.001, SE=0.0003, z=-3.343, p<0.001). The interaction between lamb lameness and strongyle FEC was significant (est= -0.007, SE=0.002, 95%CI= -0.013 to -0.001, p=0.015). Lying was positively associated with strongyle FEC for sound lambs (score 0) and lame lambs (score 1), but the association was significantly stronger in lame lambs (Figure 3.12). Ewes with a lameness score of 1 (prob=0.072, SE=0.009) were more likely than ewes with a lameness score of 0 (prob=0.183, SE=0.056) to be lying (OR=0.346, SE=0.128, 95% CI=0.134-0.896, p=0.022)(Figure 3.13).

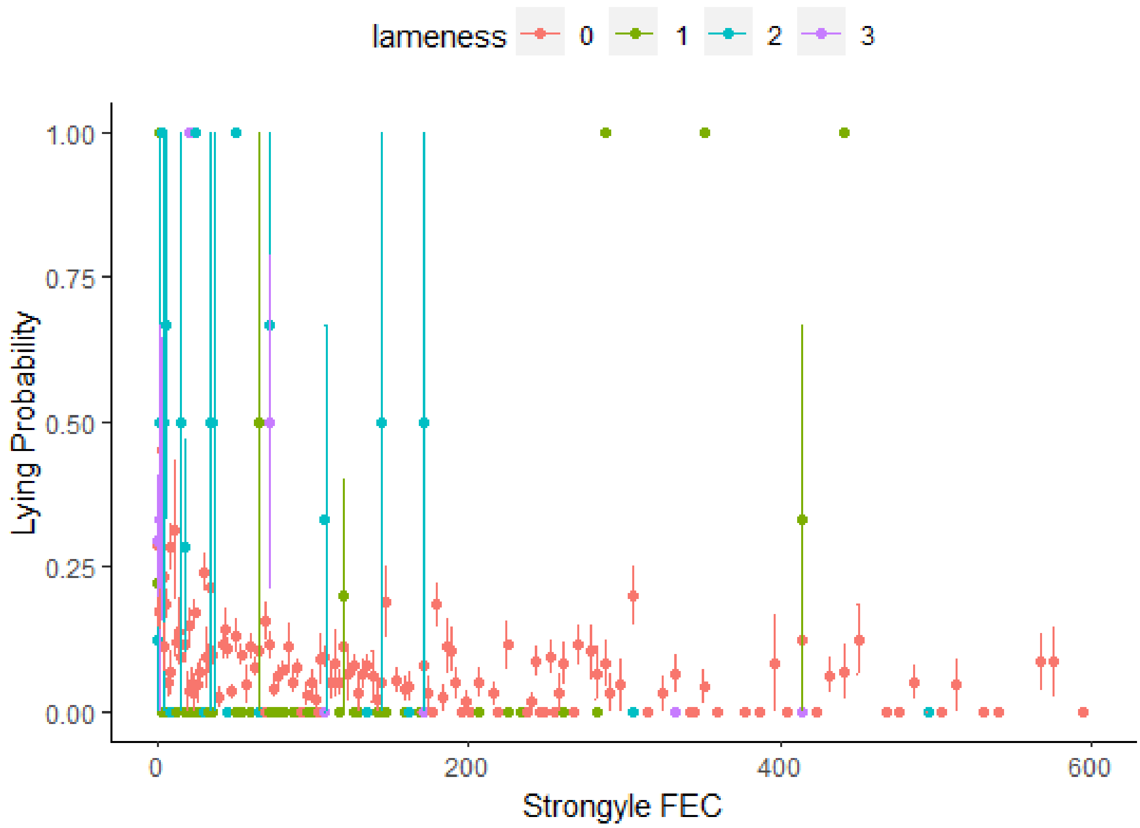

Figure 3.12. Lying probability of lambs with different strongyle faecal egg counts (FEC) and lameness scores, where 0="Movement is smooth, weight is borne equally on all 4 feet with no shortening of stride", 1="Clear shortening of the stride with obvious head nodding or flicking as the affected limb touches the ground", 2="Very obvious head nodding and not weight-bearing on the affected limb whilst moving, or lame on more than one limb", 3="Recumbent or reluctant to stand or move."

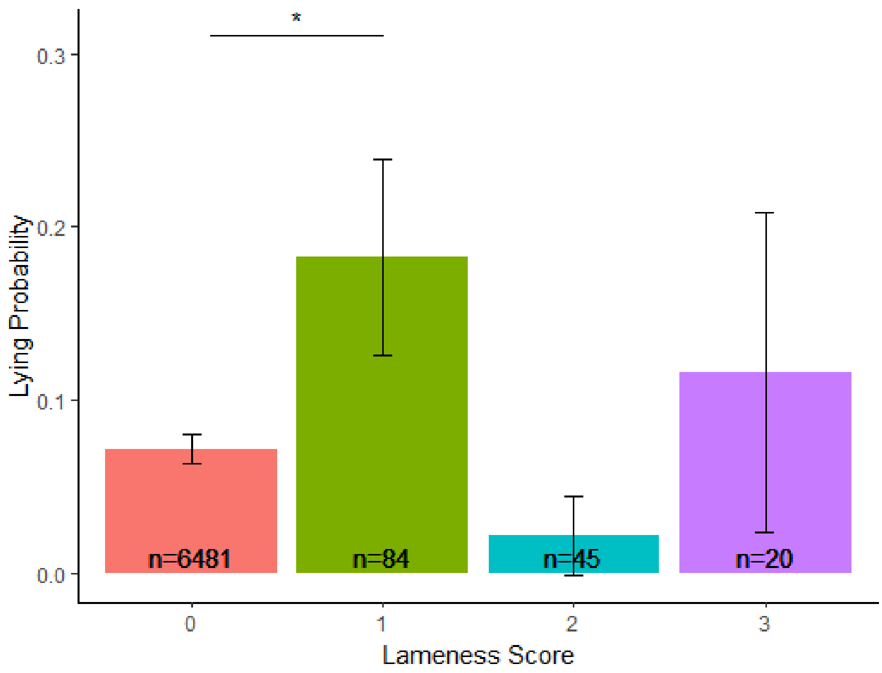

Figure 3.13. Probability of lying for ewes with different lameness scores where n=number of observations in each category, and where 0="Movement is smooth, weight is borne equally on all 4 feet with no shortening of stride", 1="Clear shortening of the stride with obvious head nodding or flicking as the affected limb touches the ground", 2="Very obvious head nodding and not weight-bearing on the affected limb whilst moving, or lame on more than one limb", 3="Recumbent or reluctant to stand or move."

3.3.4.4 Standing

There was a small yet significant positive association between *Nematodirus* FEC and lambs standing inactive (est=0.0008, SE= 0.0003, z=2.295, p=0.022), meaning that lambs with higher *Nematodirus* FEC were more likely to be observed standing inactive than those with lower FEC. Lamb standing likelihood was significantly different across lameness scores, but pairwise comparison did not reveal any significant differences. There was so little variance in the ewe standing model that it could not run.

3.3.4.5 Ruminating

There was a significant difference in ruminating likelihood for ewes with different lameness scores (p=0.037). However, the pairwise comparison did not yield significant results.

3.3.4.6 Play and sucking

Total duration of play was negatively associated with lamb *Nematodirus* FEC (est=-0.01, SE=0.01, z=-2.70, p=0.007). Number or mean duration of play bouts was not significantly associated with strongyle FEC or lameness score. Sucking behaviour was not significantly associated with any welfare scores measured.

3.3.4.7 Ewe-lamb distance

There were no significant associations between ewe-lamb distance (as a binary response variable) and lamb lameness (p=0.761), lamb strongyle FEC (p=0.679), lamb *Nematodirus* FEC (p=0.367) or any interaction terms. Similarly, ewe lameness did not impact ewe-lamb distance (p=0.408), but mastitis and ewe FEC could not be tested due to lack of variation.

3.3.5 Qualitative Behaviour Assessment

3.3.5.1 Results from 2021

The Principal Component Analysis (PCA) of lamb QBA data revealed that PC1 accounted for 32.46% of variance, PC2 accounted for 21.08% of variance, and PC3 accounted for 8.13% of variance. Cumulatively, PC1, PC2 and PC3 accounted for 61.67% of variance in the QBA data.

PC1 was considered to describe arousal levels, with terms such as Calm, Relaxed, and Content on one end and Alert, Wary and Tense on the other (Table 3.8). It was thought that PC2 described the valence of animals' affective states running from Subdued, Apathetic, and Listless to Vigorous, Bright, and Assertive (Table 3.8). It seemed that PC3 described social interaction or social response to stimulus from Apathetic and Listless to Sociable, Defensive, and Frustrated (Table 3.8).

Table 3.8. Matrix of 21 QBA terms used in lamb observations and the three principal components resulting from the Principal Component Analysis (PCA). Blue cells show the three terms with the highest positive covariances while orange cells show the three terms with the lowest negative covariances.

Term	PC1	PC2	PC3
Alert	0.270	0.044	-0.021
Active	0.240	0.237	-0.193
Relaxed	-0.327	0.052	0.212
Fearful	0.269	-0.210	0.079
Content	-0.331	0.095	0.212
Agitated	0.224	-0.149	0.257
Sociable	0.019	-0.005	0.368
Aggressive	-0.055	-0.094	0.244
Vigorous	0.182	0.356	-0.120
Subdued	0.004	-0.405	-0.141
Physically uncomfortable	0.171	-0.255	0.033
Defensive	0.102	-0.113	0.406
Calm	-0.330	-0.101	0.096
Frustrated	0.230	-0.228	0.310
Apathetic	0.032	-0.306	-0.372
Wary	0.275	-0.106	0.094
Tense	0.284	-0.159	0.110
Bright	0.211	0.319	0.046
Inquisitive	0.234	0.179	-0.015
Assertive	0.189	0.255	0.008
Listless	-0.006	-0.308	-0.373

Nematodirus FEC was positively associated with lamb loadings on PC1, thought to describe arousal (Figure 3.14) (est=0.05634, SE= 0.02485, z=2.267, p=0.023). Lambs had significantly lower loadings on the arousal dimension during the third of four weeks of data collection compared to week 4 (est=-1.3092, SE=0.454, 95%CI=-2.49 to -0.131, p=0.0229) (Figure 3.15). Week 3 loadings for all lambs were also significantly lower along the valence dimension (PC2) compared to week 4 (est=-5.01, SE=2.71, 95%CI=-6.64 to -2.45, p=0.004) (Figure 3.15). All lambs had higher loadings on the sociality dimension (PC3) during the fourth week compared to during week 2 (est=-1.501, SE=0.353, 95%CI= -2.419 to -0.5840, p<0.001) and week 3 (est= -1.384 SE=0.252, 95%CI=-2.039 to -0.7298, p <0.001) (Figure 3.15).

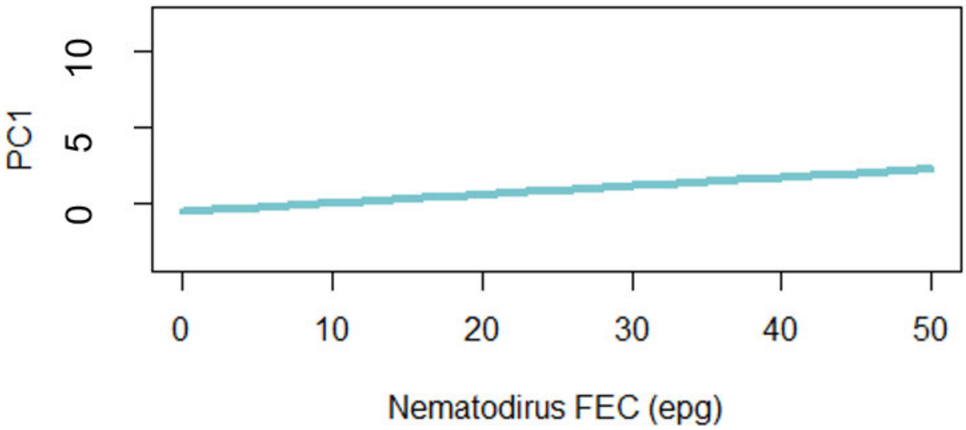

Figure 3.14. Model projection of lamb PC1 (arousal) loadings over Nematodirus FEC in eggs per gram.

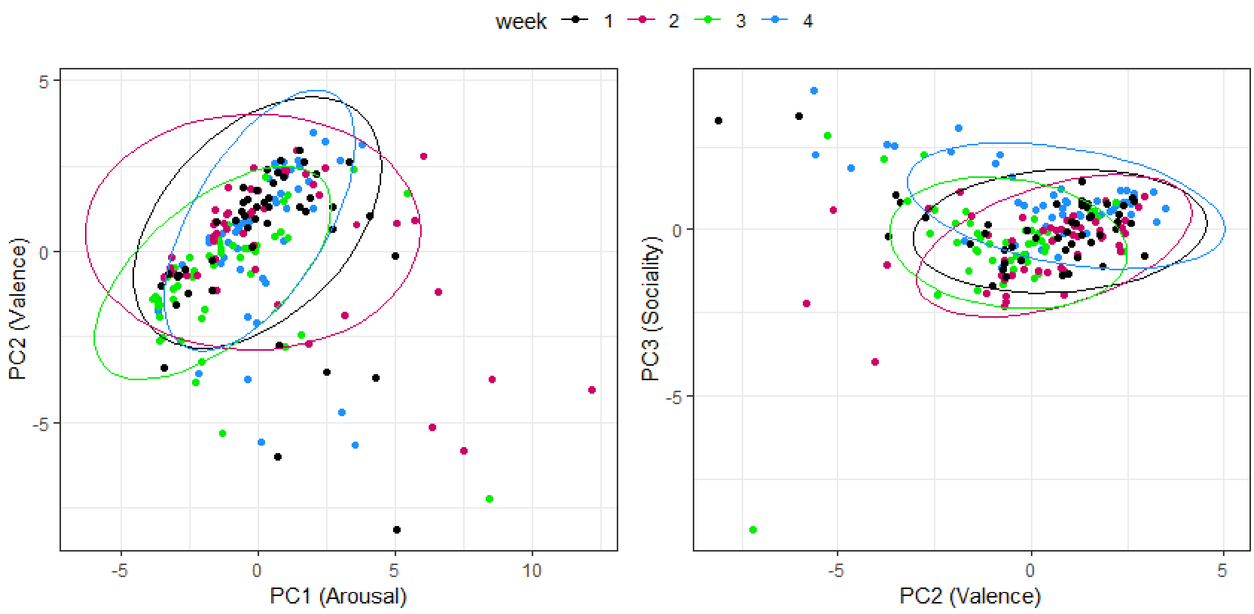

Figure 3.15. Ellipse plots of PC1 (arousal) over PC2 (valence) and PC2 (valence) over PC3 (sociality) for all lambs over the 4 weeks of QBA observations in 2021. Ellipses represent the 95% confidence interval.

3.3.5.2 Results from 2022

i. Lambs

For the lamb dataset, PC1 was considered to represent arousal with Calm and Content at one end and Bright and Vigorous at the other. It accounted for 39.7% of variance (Figure 3.14). Accounting for 14.2% of the variance, PC2 was considered to represent valence of the lambs' affective state, running from Bright and Sociable to Subdued and Agitated (Figure 3.16). The loadings on PC2were all multiplied by -1 for ease of interpretation, so that negatively valenced states (Subdued, Agitated) were on the negative side of the axes. Cumulatively, PC1 and PC2 accounted for 53.9% of variation in the data (Figure 3.16).

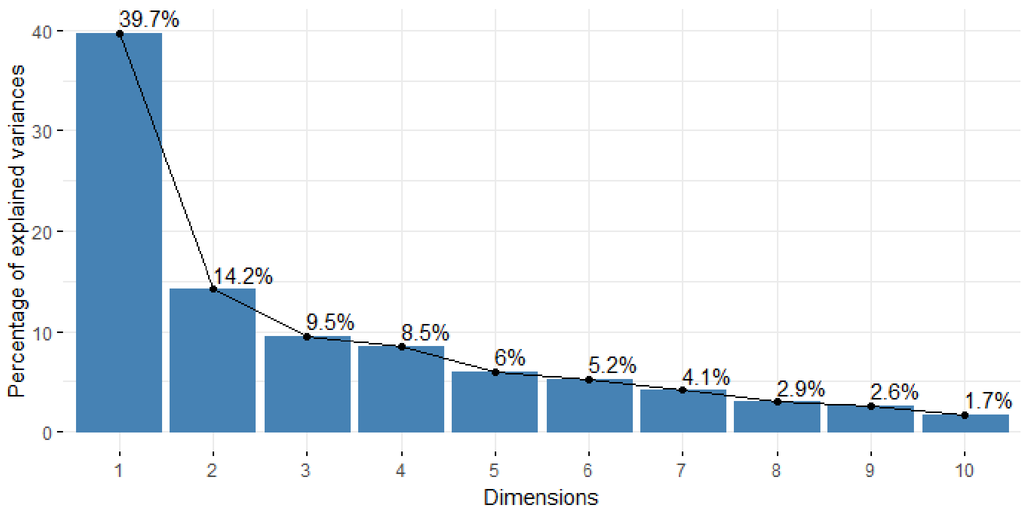

Figure 3.16. Scree plot of lamb QBA data from 2022 showing the percentage of variance explained by the first 10 dimensions.

Lambs' PC1 loadings were significantly lower before (-1.35, SE=0.31) weaning compared to after weaning (0.57, SE=0.31) (p=0.006) (Figure 3.17). Their PC1 loadings were significantly higher in the no collar group (0.63, SE=0.30) than in the collar group (-1.30, SE=0.32)(p=0.003) (Figure 3.18). On the valence dimension (PC2), lambs had significantly higher loadings after weaning (0.49, SE=1.79) than before weaning (-0.30, SE=1.78)(p=0.001) (Figure 3.17). Lamb strongyle FEC during the week before and the week after weaning was positively associated with PC2 loadings (est=0.44, SE=0.21, z=2.08, p=0.038), meaning higher FEC were associated with higher valence loadings (Figure 3.19). There was moderate repeatability of ranks for lambs before and after weaning along PC1 (W=0.48) and PC2 (W=0.48), meaning that some lambs with the highest and lowest loadings along PC1 and PC2 before weaning also held those positions after weaning.

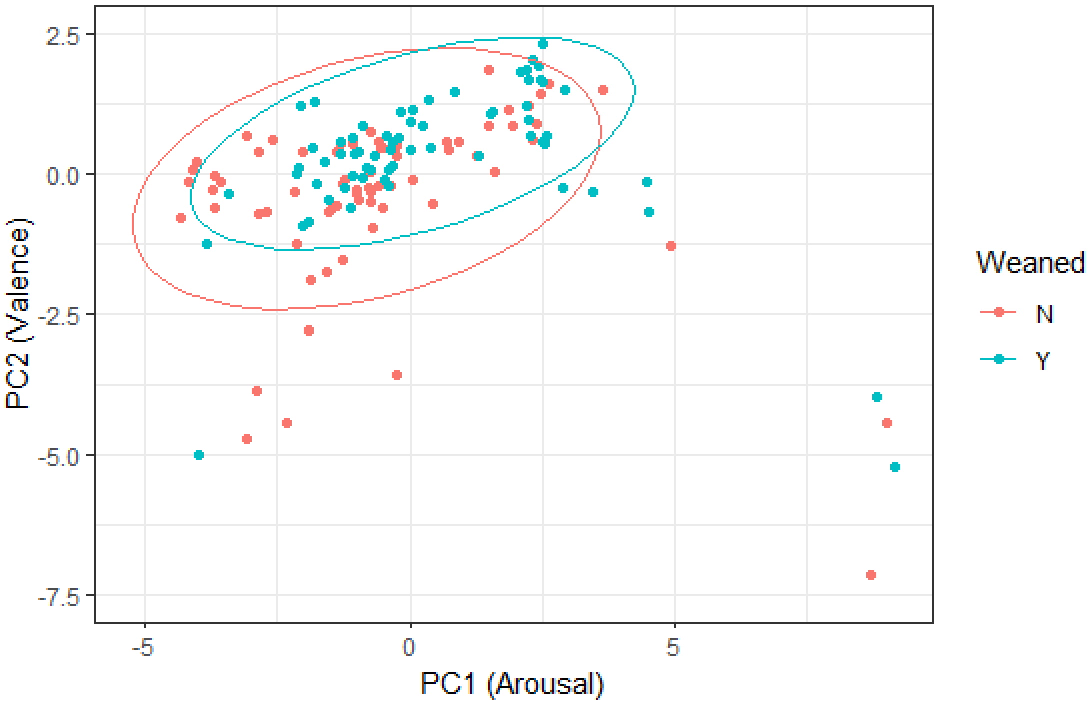

*Figure 3.17. Lamb loadings along PC1 and PC2 (transformed by *-1 for ease of graphing) before (black, N) and after (pink, Y) weaning.*

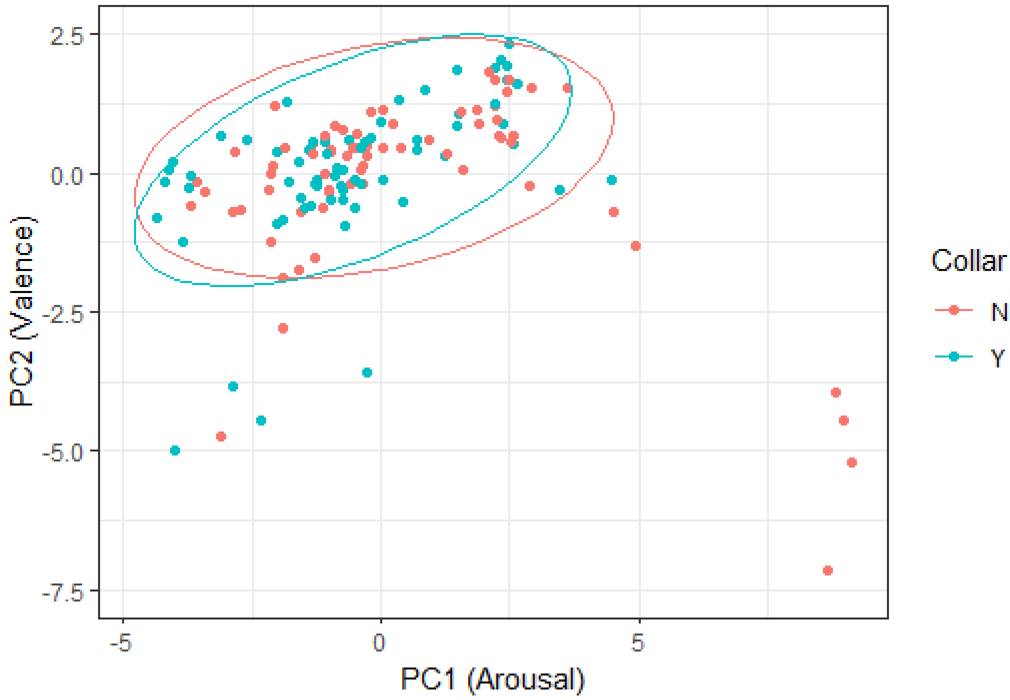

*Figure 3.18. Lamb loadings along PC1 and PC2 (transformed by *-1 for ease of graphing) without collars (pink, N) and with collars (blue, Y).*

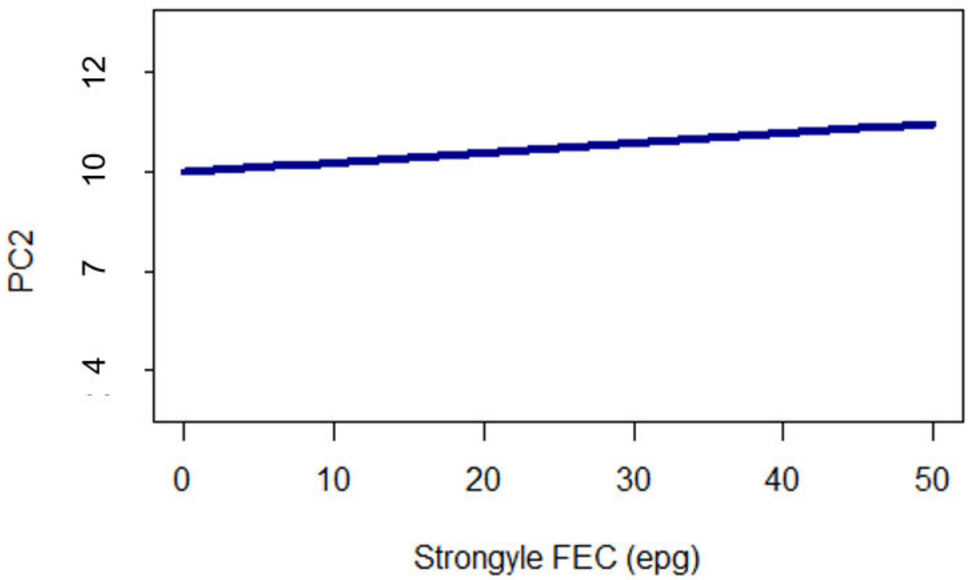

*Figure 3.19. Model projection of lamb loadings along PC2 (transformed by *-1 for ease of graphing) over strongyle faecal egg count (FEC) in eggs per gram (epg).*

ii. Ewes

For ewes, PC1 accounted for 40.5% of variance in the data, and was thought to represent arousal, running from Bright, Vigorous and Agitated to Relaxed to Content and Calm. PC1's loadings were all multiplied by -1 for ease of interpretation, so that more aroused states (Bright, Vigorous) were on the axis' positive side. Accounting for 14.1% of the variance, PC2 possibly described valence of ewe affective state going from Subdued and Physically uncomfortable to Content and Bright. In ewes, no significant differences existed between PC1 and PC2 loadings before and after weaning (Figure 3.20) or between ewes with and without collars. However, ewe strongyle FEC was negatively associated with PC1 loadings (est= -0.119, SE=0.051, z= -2.32, p=0.020), meaning higher FEC were associated with lower arousal loadings (Figure 3.21). There was moderate repeatability of ranks for ewes before and after weaning along PC1 (W=0.52) and PC2 (W=0.46).

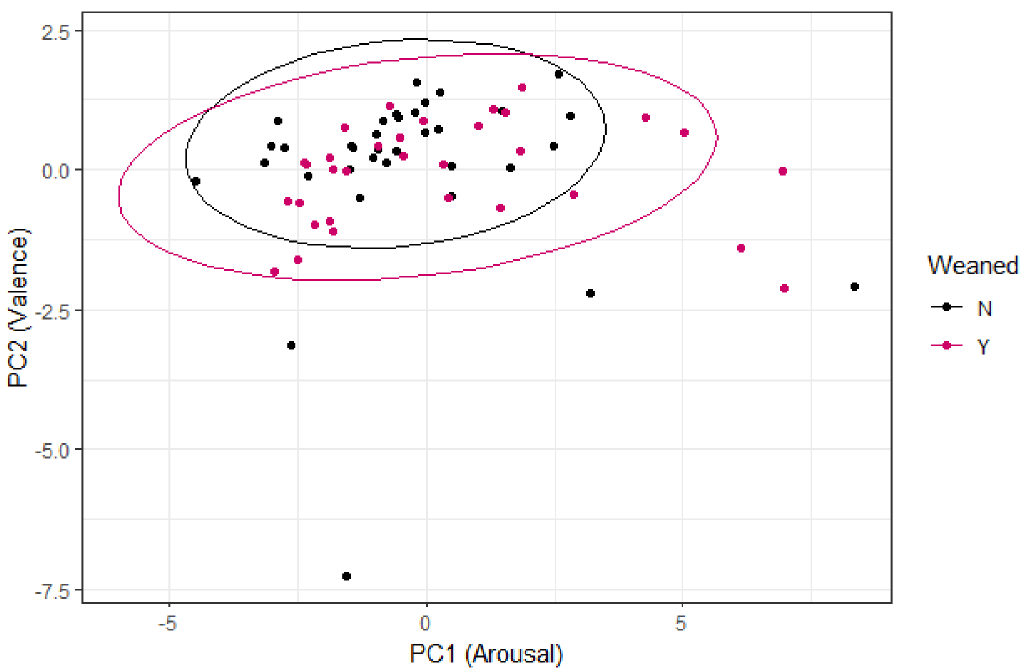

*Figure 3.20. Ewe loadings along PC1 (transformed by *-1 for ease of graphing) and PC2 before (black, N) and after (pink, Y) weaning.*

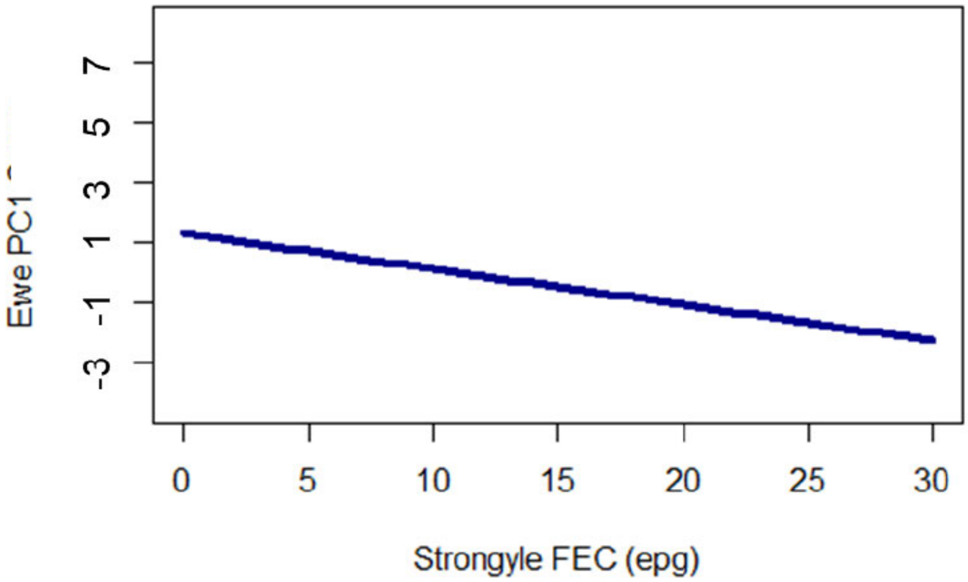

*Figure 3.21. Model predictions of ewe loadings along PC1 (transformed by *-1 for ease of graphing) over ewe strongyle faecal egg count (FEC) in eggs per gram (epg).*

3.4 Discussion

The purpose of this study was to identify early behavioural indicators of GI parasitism, lameness and mastitis through behavioural observations, with the aim of eventually using technological sensors to record these behavioural indicators. In lambs, lameness was associated with a reduction in grazing behaviour likelihood and possibly affected standing behaviour. Parasitism impacted lamb grazing, locomotion, lying and standing in various ways according to the parasite species. Mastitis occurred too rarely for analysis to occur. Some temporal differences were recorded through QBA on the arousal and sociability dimensions in 2021 for lambs and suggested a more positive valence of mental state after weaning in lambs.

The lack of significant differences in FEC between parasitology treatment groups in 2021 rendered analysis across treatment groups unnecessary. Therefore, where possible, models were built using FEC as a covariate rather than parasitology treatment group as a fixed effect. *Nematodirus* FEC of HP and LP lambs was likely not significantly different from each other because of timing; the study began after the peak of seasonal *Nematodirus* infection had already passed, and all lambs were naturally recovering from infection, regardless of treatment group. Adult ewes have developed relative tolerance and immune responses to nematode infections over years of exposure, resulting in their FEC remaining relatively stable regardless of treatment group (McRae et al., 2015; Smith et al., 1985).

Ewe welfare scores recorded a mild impact of the welfare conditions being monitored, leading to low variation in the data and difficulty modelling any relationships involving the welfare indicators. This results from relying on natural infection, which is a good way to mimic normal commercial conditions and to avoid imposing high levels of controlled infection onto animals. However, natural infection does not lead to reliable numbers of infections and often requires large amounts of animals to reach statistical significance (Colby et al., 2017). Repeating this study on animals already undergoing experimental trials where inoculation with diseases is planned could be a way of addressing this issue while not increasing the number of animals needed. Otherwise, studies on pastures known to be infected with high levels of *Dichelobacter nodosus*, the causal agent for footrot, may help ensure higher levels of variation in lameness scores, for example. However, overall ewe lameness in this study was close to the national average of 3.2% (Best et al., 2020). Some records of ewe weight were very low in 2021, with some individuals recorded as weighing 25kg. These are likely weighing errors due to weigh head malfunctions, which occasionally occurred.

In lambs, lameness was similarly rare. Breathing, fleece and injury scores were rendered unusable in models due to the vast majority of lambs never scoring a 1. Natural infection and the high levels of staff attention these animals received could explain their relative lack of welfare problems. The range of weights was much greater in 2022 due to monitoring occurring from the third week of life to the end of the grazing season, whereas the first weighing event in 2021 occurred when the lambs were 8 weeks old. As for parasitism, sheep develop a significant protective immunity to the larval challenges they are regularly exposed to by the age of 10-12 months (McRae et al., 2015). It was therefore unsurprising that the ewes' FEC remained low throughout both years while the lambs' FEC were much more variable. The wider range of ewe FEC in 2022 may result from the higher number of samples taken and sampling beginning earlier, meaning the periparturient relaxation of ewes' immunity, which occurs around lambing, could have been captured (Gibson, 1973; Nisbet et al., 2016).

Positive or negative associations between behaviours and day of experiment resulted from the natural change in behavioural expressions of lambs over time and the fact that our ethogram was constructed so that the behaviours were mutually exclusive. For example, as lambs aged they were significantly less likely to be observed performing locomotion. This is probably because they spent more time grazing as they grew older and grass came to replace milk in their diet (Hadjipieris et al., 1965). Differences between the two years are most likely due to the fact that recording occurred over a much longer period in 2022, capturing more behaviours at various times throughout the season. For example, lambs were observed ruminating more often in 2022 presumably because there were more chances to record this behaviour due to the more frequent observations throughout the lambs' entire lives, compared to 2021, when the lambs were only observed for four weeks at eight weeks old, then again for four weeks at 16 weeks of age. Additionally, it is possible that differences in weather across the years affected the behaviours observed. In 2022, there were long period of unusually hot and dry weather, which may explain why more ruminating behaviour was recorded in ewes and lambs, for example, as the sheep would have sought shelter and rest in the hot weather.

Play, whether social or locomotor, was rarely recorded. Only 7 of 48 lambs displayed any play behaviour during the entirety of behaviour samples collected. It is possible that at 8 weeks of age when the observations began, the lambs were too old to be displaying much play behaviour. In a study on the ontogeny of play in lambs, play peaked during the second and third weeks of life, then again around the eighth and ninth weeks, before decreasing significantly (Sachs & Harris, 1978). They were observing lambs of various lowland breeds (Dorset, Shropshire, and Southdown) for 15 minutes weekly (Sachs & Harris, 1978). Given that the observations in this study were 30 minutes and did cover the eighth and ninth weeks of life, more play behaviour was expected. Behaviour samples were not repeated in 2022 after concluding that they would likely lead to few results for the large amount of labour they require. Already seeing the low levels of variation in the behavioural and welfare data at the end of the 2021 trial, it was decided that more scan samples might be more useful in collecting more varied data.

Ewe-lamb distance increased as lambs grew older and spent more time away from their dams. This reflects the move from the postpartum period, when ewes remain very close to their lambs, to a time where lambs are more independent (Dwyer & Lawrence, 2005; Sohi et al., 2017). Only three of the same days were observed in 2021 and 2022, making it difficult to compare daily ewe-lamb distance directly across years. However, comparison between ewe-lamb distance data from June 2021, where the maximum value is 50m, to June 2022, where the maximum value is 120m, is possible. The reason behind this significant different in ewe-lamb distance between years is unclear. It may be because ewes in 2022 were primiparous, compared to the 2021 ewes, who were multiparous. Primiparous ewes prevent their lambs from sucking more often and are more likely to butt or withdraw from their lambs than more experienced ewes, who display longer durations of sucking bouts (Dwyer & Lawrence, 2000b; Ekiz et al., 2007; Karaca et al., 2023). These behavioural differences may explain the larger distance between primiparous ewes and their lambs. However, it must be noted that these findings were reported in studies that only observed behaviour on the day of lambing (Dwyer & Lawrence, 2000b; Ekiz et al., 2007; Karaca et al., 2023). The distance between ewes and lambs was not affected by welfare challenges to either party, meaning this measurement did not act as a useful proxy for welfare in this study. Studies with more variation in welfare data, for example through higher recorded levels of lameness or mastitis, are needed to conclude on this measure's functionality as an indicator of welfare.

Lame lambs were less likely to be grazing than sound lambs, presumably because it was painful to do so. In a previous study using biologgers for the automatic detection of lame ewes, lame animals were less likely to graze (Lewis et al., 2023). These results strengthen the case for a reduction in grazing behaviour to be used as an indicator of lameness. Even mild lameness, recorded with a score of 1, impacted the likelihood of lambs grazing. This reinforces the calls for early treatment found in many sheep lameness studies (FAWC, 2011; Green et al., 2012, 2014). Recent studies suggest that lame ewes and lambs are more inactive than sound animals (Lewis et al., 2023). The results of the present study did find this in adult sheep: ewes with a lameness score of 0 were less likely than ewes scoring 1 to be lying down. The lack of pairwise results comparing lameness groups' standing likelihood rendered it impossible to test whether lame lambs in this study were more inactive than sound lambs. Sheep graze in social groups and small social groups display more vigilant behaviours and forage less (Dumont & Boissy, 2000; Sevi et al., 1999). Lame sheep who spend more time lying down could create smaller groups grazing together and impact the entire social group's foraging efficiency (Morris et al., 2022). The inability to graze may lead to frustration, and reduced grazing behaviour could affect lamb welfare through not being able to meet their nutritional needs (Temple & Manteca, 2020). In indoor systems, grazing restriction can lead to abnormal and aggressive behaviours like wool-pulling (Parés et al., 2023), although motivations may differ between the involuntary restriction imposed in the cited indoor study and sickness behaviour in the present study.

Nematodirus infection negatively affected the likelihood of lying, and conversely increased the likelihood of standing inactive in lambs. Standing alert postures may represent discomfort behaviour, where the lamb is too uncomfortable to lie and rest but has no desire to walk and graze. This increase in standing inactive could contribute to the reduced amount of feeding activity reported in the present study as well as in parasitised ruminants in previous studies (Hutchings et al., 2000; Kyriazakis et al., 1998). Mastitic cows spent less time lying and more time standing to avoid putting pressure on the mastitic udder quarter (Siivonen et al., 2011). The lambs' increased standing in this study could similarly be a sign of abdominal discomfort caused by the abomasal lesions being inflicted by parasite larvae in the early stages of infection. This reflects findings from the indoor pilot trial in Chapter 2, where lambs parasitised with the gastrointestinal nematode *Teladorsagia circumcincta* were more likely to be observed standing inactive during scan sampling than unparasitised lambs. Additionally, I hypothesise that the lambs' awareness of their illness and thus reduced fitness could lead to an increase in vigilance as a prey species survival mechanism. Grazing behaviour increasing with strongyle FEC but decreasing with *Nematodirus* FEC could be explained by the fact that in the UK, *Nematodirus* affects young lambs at the start of their lives, when they are less likely to be grazing due to their nutrition mainly coming from their dam's milk, whereas strongyle infections tend to occur later in the season, when lambs have been weaned and spend more time grazing, regardless of infection status (Stubbings et al., 2020). The temporal patterns of infection in this study matched these expectations.

Increased *Nematodirus* FEC was linked to a decrease in the total duration of play bouts. Although play is a notoriously flexible behaviour with a lot of within-species variation, it is interpreted as an indicator of positive welfare (Brown et al., 2015; Held & Špinka, 2011; Mellor, 2012). Reductions in play have been reported to accompany reduced food availability; play decreased in bottle-fed deer fawns whose intake was reduced by 33% (Muller-Schwarze et al., 1982) and calves reduced their playful running after milk availability was reduced (Krachun et al., 2010). It is possible that parasitised lambs displaying parasite-induced anorexia reduced their play behaviour as their fitness and welfare is compromised by this reduction in feed intake and disease. This would align with the theory behind sickness behaviour, where energy demanding behaviours with long term benefits like play decrease, while resting behaviours tend to increase (Ghai et al., 2015). Through decreased foraging, socializing and mating, the cost of this sickness behaviour can be relatively high (Hart, 1988). But it also increases the chances of fighting off the pathogen by diverting energy away from energetically expensive behaviours and towards the immune response (Hart, 1988). However, feed intake was not measured in this study, rendering it impossible to reach a conclusion on this. Calls for the potential of play behaviour as an indicator of parasitism in grazing ruminants to be studied have been published (Bricarello et al., 2023). However, in this study, lamb *Nematodirus* FEC peaked between eight and 12 weeks of age, which may be too late to link it with play behaviour, which tends to drop off after eight to nine weeks of age (Sachs & Harris, 1978). The present study's findings add to the existing knowledge and the need for further research.

The significant effect of the interaction between lameness and strongyle parasitism on locomotion and lying are difficult to interpret. It may be the result of low variation in the dataset and rarity of lambs scoring 2 or 3 on the lameness scale. However, it could indicate that lameness score 1 is more impactful than it seems, seeing as that is the score at which behaviour is the most altered in this study. In the case of lying behaviour, the significant interaction potentially illustrates the impact of cumulative harms that GI parasitism and lameness can have. Subclinically parasitised (FEC < 400 epg) but sound lambs were only slightly more likely to be observed lying than healthy lambs, while subclinically parasitised and lame lambs were even more likely to be lying. The importance of monitoring cumulative stress and welfare issues is often discussed in transport studies, as these follow animals over relatively long periods of time, but the concept is equally important in on-farm welfare assessments (Hall & Bradshaw, 1998; Willis et al., 2021). When examining the data, only one lamb scored 3 on the lameness scale, and it had the highest likelihood of standing compared to lambs scoring 1 or 2. Although this is not statistically significant, it may provide initial anecdotal evidence that lameness could be detected by increased occurrence of standing inactive behaviour in lambs.

Due to differences in timing of observations across years, the QBA analysis served different purposes in 2021 and 2022. In 2021, I assessed changes in affective state over time across the four weeks of observation. In the 2022 analysis, it was more useful to use weaning as a known welfare challenge and observe how the sheep responded, seeing as no disease-related welfare challenges were applicable to a sufficient number of animals to compare their QBA scores across lameness scores, for example. The higher loadings along the sociality dimension for all lambs in week 4 of observation in 2021 could be due to lambs aging and interacting with each other in peer groups more than with their dams. The lambs were 11 weeks old in week 4. The ewe-lamb distance has been reported to increase up to 100 metres during this period where lambs socialise more with peers (Arnold & Grassia, 1985; Pickup & Dwyer, 2011). The lower loadings on the arousal and valence dimensions during week 3 could be explained by the lambs spending most of their time grazing for the first time in their lives. Alternatively, it could reflect an unrecorded health or welfare challenge that affected the lambs during week 3. Weather records indicated that the four days of QBA observation had similarly mild temperatures and little precipitation (*Edinburgh Historical Weather (United Kingdom) - Weather Spark*, 2023). Week 3 had higher wind speeds than other weeks, at 20km/hour, possibly contributing to the lambs' lower arousal and valence loadings (*Edinburgh Historical Weather (United Kingdom) - Weather Spark*, 2023). The positive association between lamb *Nematodirus* FEC and arousal loadings is surprising, given that FEC remained low (below 50 epg) throughout the QBA observation period. The possibility of higher arousal loadings for lambs with higher FEC vaguely echoes the behavioural results presented in this chapter and in Chapter 2, where parasitised lambs were more likely to stand inactive. A standing inactive posture could be indicative of a higher state of arousal or vigilance. Trials with a higher parasite burden are needed to further test the possible relationship between FEC and QBA.

In 2022, weaning did not have a significant effect on the affective states of ewes but lamb loadings on PC1 (arousal) and PC2 (valence) were higher the week after weaning than the week before. Commercial weaning practices tend to provoke stress-related behavioural responses in lambs and their dams, which could explain the higher arousal loadings, but lower valence loadings would have been expected (Freitas-de-Melo et al., 2022). Weaning triggers ewes and lambs to increase their pacing and the number of vocalisations they make (Cockram et al., 1993; Damián et al., 2013; Freitas-de-Melo & Ungerfeld, 2016). These behavioural changes peak a few hours after weaning but remain for up to 2-3 days, which was when the post-weaning QBA observations were conducted (Freitas-de-Melo et al., 2013, 2017, 2019). Since these are energetically demanding behaviours, it would be expected that lambs and ewes would appear more stressed and tired in the days after weaning (Weary & Fraser, 1995). It was rainier and cooler on the observation day before weaning compared to the warm day of observations after weaning, which may have impacted sheep behaviour interpretation (*Edinburgh Historical Weather (United Kingdom) - Weather Spark*, 2023).

The higher arousal loadings of lambs wearing collars compared to lambs without collars may be cause for concern and is fully explored in Chapter 4. Lamb strongyle FEC being positively associated with PC2 loadings was surprising, as an increased worm burden would have been expected to have a negative impact on lamb mental state. However, FEC remained very low (below 50 epg), meaning it is possible that the negative impact of strongyle parasitism is not yet present at this low level of infection. Another possibility is that the QBA picked up a different, unrecorded measure that was affecting the lambs at that time.

The only significant relationship between welfare measures and QBA results found in ewes in 2022 was a negative association between strongyle FEC and PC1 loadings. This is the opposite relationship that was found in 2021 between lamb *Nematodirus* FEC and PC1 loadings. If the hypothesis that increased FEC is linked with increased arousal and standing behaviour in lambs is to be accepted, then these results could indicate that this relationship is not seen in adult sheep, who have developed robust immunity against the worms, as demonstrated by ewe strongyle FEC not exceeding 30 epg in the QBA observation period.

There was repeatability of ranks of individual loadings for ewes and lambs along both dimensions in 2022. This means that certain animals with the highest and lowest loadings before weaning also held these positions after weaning. This indicates that

QBA may have measured long-term mood patterns rather than capturing a fleeting emotion in the animals at the time of observation (Kremer et al., 2020).

The initial hypothesis that behavioural change would occur before clinical signs of disease were visible was unable to be tested due to analysis challenges. The difficulty lay in identifying exactly when bouts of lameness, parasitism or mastitis began and ended, and doing so for a large number of animals. Notably, this was a challenge because relatively few animals experienced any kind of clinical challenges. Functional time series analysis could identify patterns and temporal relationships between behaviour and welfare. If this kind of analysis was successful, time series forecasting could develop predictive models based on the findings.

3.5 Conclusions

Understanding behavioural changes brought on by diseases in sheep could allow us to detect and treat them early, improving animal health and welfare. I found that lamb likelihood of standing behaviour was positively associated with parasite FEC while lying and grazing behaviour decreased with increasing FEC. Play behaviour decreased as *Nematodirus* infection levels rose and lame lambs grazed less than sound lambs. These behavioural indicators have the potential to act as early signals of the studied diseases and compromised welfare. Qualitative results found temporal differences in behavioural expression of lambs and associations between parasitism and mental state that require further research. These finding contribute to the research describing sickness behaviour in sheep.

Chapter 4. Validation of accelerometers for determining sheep behaviour and the welfare impacts of wearing collars containing technology.

4.1 Introduction

PLF technology has the potential to improve farm animal health and welfare in several ways. For example, Global Positioning Systems (GPS) can alert farmers to cows calving, Bluetooth connected weigh crates can identify lambs needing anthelmintic treatment, and custom-made accelerometers can detect lameness in sheep before clinical signs are visible (García García et al., 2023; Kaler et al., 2020; Morgan-Davies et al., 2018). In extensive management systems, monitoring sheep in unfenced pastures is time-consuming and labour intensive, especially as the farming labour force dwindles (Buller et al., 2020; Haigh, 2010; Morris et al., 2012). Technology offers increased opportunities for monitoring leading to more biometric data collected from individual animals (Morris et al., 2012). In turn, PLF can help in decision-making and improve sheep health and welfare (Haigh, 2010; Buller et al., 2020). However, welfare impact studies and validation studies are required before commercialisation to ensure animal welfare is respected and prioritised while reliable information is being collected.

The risk of wearable technology altering animal behaviour and skewing data has long been recognised, but these behavioural changes can also indicate direct negative effects on animal welfare, which must be taken into account during technology development (Ropert-Coudert & Wilson, 2005; Tuyttens et al., 2022). Welfare impact studies collect data on changes in welfare indicators when the technology is used. They can identify and address issues before they are widely spread. For example, group level technology such as automatic feeders or milking robots can change the social dynamic of a system or increase the risk of disease transmission (Anil et al., 2006; Hovinen et al., 2009; Kirchner et al., 2012). Studies report that sows compete over access to automatic feeders, especially when queueing occurs, increasing aggression levels and decreasing body condition among low-ranking individuals (Anil et al., 2006; Kirchner et al., 2012; Remience et al., 2008). Others have found that dairy cow somatic cell count increases in the first year after the installation of automatic milking systems, as the cows adjust to irregular milking times where teats don't fully recover over short milking intervals and longer intervals cause bacteria to multiply (Hovinen et al., 2009; Hovinen & Pyörälä, 2011). On an individual basis, wearable sensors risk causing physical damage to animals (Tuyttens et al., 2022). For example, a 36-gram backpack containing a tag for a tracking system in broilers was reported to decrease walking behaviour and increase pecking behaviour directed at the tag-wearing chickens by other birds in the first week (Stadig et al., 2018). Although behaviour returned to normal after the first week, the tags went on to cause a significant increase in red mite colonisation (Tuyttens et al., 2022). Standard cattle ear tags can cause injuries (Johnston & Edwards, 1996), so it is likely that any technology-laden ear tags would have the same risks, or more important risks as they may be heavier, for example. Welfare impact studies are rarely published before the commercialisation of products, as they can be costly and time-consuming, and they may result in delays in the arrival of tools to market (Tuyttens et al., 2022).

PLF technology also needs to be independently validated to minimise false positives, false negatives and inaccurate monitoring in general. However, review papers have identified a lack of published studies: only 5% of human wearable technologies have been scientifically validated, 14% of dairy cow PLF systems for welfare monitoring have external validation trials available, and 23% of published studies on PLF in pigs were formally validated (Larsen et al., 2021; Peake et al., 2018; Stygar et al., 2021). Companies developing PLF tools are under economic pressure to protect their product development processes and commercialise products quickly, which may partly explain the lack of publicly available validation studies (Tuyttens et al., 2022). Despite this, examples of rigorous validation studies exist: the use of IceQube sensor (IceRobotics, Edinburgh, UK) for recording postures and step numbers in lambs has been validated (Högberg et al., 2020). Bluetooth technology has been validated for monitoring weaned lamb location in the field (Walker et al., 2023). One thing these two validation studies have in common is that they test the use of a technology in a specific setting for a precise use on a particular species of a defined age (Högberg et al., 2020; Walker et al., 2023). This level of detail and rigour in validation studies must continue to be encouraged as new PLF tools are developed and applied in new settings.

Tri-axial accelerometers have received a lot of attention due to their small size, long battery life and wide range of applications (Herlin et al., 2021). They record the location of the device on an x, y and z axis in space, which when observed over a period of time, describes movement direction and intensity. They have been shown to detect lameness and parasitism in sheep and oestrus in cattle, along with a number of other conditions and behaviours (Benaissa et al., 2020; Herlin et al., 2021; Kaler et al., 2020; Morris et al., 2022). They can be worn on collars or harnesses on the back or the tail, or incorporated into ear tags, pedometers or boluses (Herlin et al., 2021). Examples of four commercially available accelerometers specifically marketed for ruminants include: RumiWatch by Itin+Hoch (Liestal, Switzerland), IceTag by IceRobotics (Edinburgh, UK), CowManager SesnOor by Agis Automatisering (Harmelen, Netherlands) and Afimilk (Israel). These were all originally developed for cattle, though some of IceRobotics' tools have since been tested and validated for sheep, sows and horses (DuBois et al., 2015; Högberg et al., 2020; Morris et al., 2022; Ringgenberg et al., 2010). A plethora of other brands of accelerometers are available and marketed for different uses. The AX3 accelerometer (Axivity Ltd., Newcastle Upon Tyne, UK) is marketed for human activity monitoring and has mostly been used and validated in this context to date (Clarke et al., 2017; Kongsvold, 2016). Its limitations include the fact that the devices must be removed from the animals to download data at regular intervals, which increases the risk of data loss and time drift, as with any tool that is recording live information. This also increases animal handling occurrences, which can be stressful (Tuyttens et al., 2022). Additionally, the AX3's battery life in the present study was approximately four weeks when running continuously. This would have to be substantially extended for any future commercial use or an alternate power source would need to be included in the design. However, its small and light design (23x32.5x7.6mm, 11g) makes it a good candidate for livestock research, especially on smaller animals such as young lambs. For example, the AX3 has been used to monitor grazing and ruminating behaviour in dairy cows, to study the kinetic energy harvesting potential of their locomotion, and to detect oestrus (Benaissa et al., 2020, 2022; Blazevic et al., 2022).

Using welfare data collected over two grazing seasons, this study aimed to determine whether wearing a collar containing the AX3 accelerometer had welfare implications for ewes and lambs. I hypothesised that the collars would not impact ewe or lamb welfare as previous studies have not reported any issues, although testing is not always clearly described (Barwick et al., 2018; Burgunder et al., 2018; Mansbridge et al., 2018). I also aimed to validate the AX3 accelerometers for detection of behavioural states and activity levels in ewes and lambs. I hypothesised that it would differentiate between behaviours, or at least between active and inactive behaviour patterns.

4.2 Methods

4.2.1 Ethical approval

Ethical approval was obtained from the Moredun Research Institute's AWERB (Animal Welfare and Ethical Review Board (Trial 20-21 and Trial 18-22, project number PPL P95890EC1I)).

4.2.2 Animals

This study was conducted on the same animals described in Chapter 3. Focal behavioural observations for AX3 validation were conducted between July 21st and August 10th 2021. Weaning occurred in the afternoon of August 9th 2021, meaning that ewes and lambs were in the same paddock for the first three observations, but were separate post-weaning for the last set of observations. Welfare and behavioural data were collected over eight weeks (2 blocks of 4 weeks) in 2021 and sixteen weeks (4 blocks of 4 weeks) in 2022.

In both years, the ewes and their lambs grazed on three adjoining 1-hectare paddocks before weaning. The gates separating the paddocks were left open to allow movement between them. The rectangular paddocks all contain drinkers, trees and large dead branches which provide shade and shelter. After weaning, the lambs were kept in a separate paddock from the ewes, so that visual contact was no longer possible though the ewes and lambs could hear each other vocalizing.

4.2.3 Experimental Design

Over both years, the animals were divided into two treatment groups. Half of ewes wore collars containing an AX3 accelerometer (Axivity Ltd, Newcastle Upon Tyne, UK), a Feasybeacon Bluetooth beacon (Feasycom, Shenzhen, China) and a i-gotU GT-120B GNSS Data logger (MobileAction, New Taipei City, Taiwan). The ewe collars were made from polyester running belts purchased online and are described further in 4.2.4.3. The other half did not wear any collars. Each ewe collar containing technology weighed approximately 300 grams. One lamb from all pairs of twins wore a homemade elasticated fabric collar with Velcro attachments, containing an AX3 and Feasybeacon sewn into a pocket on the lamb collars, further described in 4.2.4.2. Each lamb collar containing technology weighed approximately 40 grams. Ewe and lamb allocation to treatment group was randomized and balanced for ewe weight and lamb sex. The current study only reports on AX3 findings, while the other two PLF tools were used for experiments separate from this thesis.

4.2.4 Data Collection

4.2.4.1 Welfare monitoring

Welfare assessment was conducted as described in section 3.2.4.1 of Chapter 3, using the scores described in Table 3.1, in 2021 and 2022.

4.2.4.2 Behavioural observations for AX3 validation

Observations were carried out by two observers (MR, HM) using 20-minute focal samples of 6 individual ewes and 6 lambs over four days. This led to 480 minutes of validation observations for ewes, and 480 minutes for lambs. During every observation, focal samples were conducted on 6 ewes and 6 lambs chosen at random upon entering the paddock to capture the widest range of behavioural expression as possible. The ethogram in Table 4.1 was used to record behaviours and a digital GPS clock was used to record the times at which behaviours were performed. Some were only observed once while others were repeatedly sampled. This resulted in ten ewes and 12 lambs being observed over the four validation observations. Data from the AX3 were obtained from all animals except one ewe whose AX3 malfunctioned. On July 21st and August 3rd 2021, observations were noted on paper data sheets before being transcribed into Microsoft Excel as the video camera described below was not yet available. On August 8th and 9th 2021, focal observations were recorded with a Canon HD CMOS Pro video camera with a Canon WD-H58W wide-angle lens (Canon, Melville, USA), annotated using The Observer XT15 (Noldus Information Technology, Wageningen, Netherlands) and data was exported to Microsoft Excel.

Table 4.1. Ethogram and abbreviations for focal observations of ewes and lambs.

Behaviour	Description
Grazing/Drinking* (g)	Chewing or obtaining grass or foliage, or water from trough, with head down below the shoulders within 10cm of the ground while lifting one or more feet off the ground and moving forward or with four feet not leaving the ground.
Locomotion (lo)	Moving feet, leading to motion in any direction for more than 2 seconds.
Lying (ly)	Animal's body is touching the ground from shoulder to back end, neck and head touching the ground or upright, absence of other behaviour
Standing (st)	Remaining still in a posture where head is raised above the level of the back, up on all four legs.
Standing rumination (sr)	Remaining still in a posture where head is raised above the level of the back, weight placed on all four legs, feed being regurgitated into mouth and chewing
Play (pl)	Lamb running with no obvious destination to reach, jumping or pivoting for no obvious reason, butting or mounting another lamb
Social behaviour (sb)	Being in active physical contact with another sheep, including nudging, nuzzling, or nosing
Scratching (s)	Standing and rubbing body or head against fencing, tree or water trough or nosing a part of the body repeatedly.
Ruminating (r)	Resting with whole body on ground off all four feet, head up above shoulder level, regurgitation or chewing by moving bottom jaw for more than 5 seconds
Sucking (su)	Lamb's mouth in contact with or within 10 cm of the ewe's udder for longer than 2 seconds
Unclear (u)	Animal's behaviour is concealed by a visual barrier e.g. bush or another ewe/lamb.

* Drinking behaviour is extremely rare but it was deemed appropriate to combine with eating since the motivations driving both behaviours as well as the effects of disease on them may be similar.

4.2.4.3 Collars

Ewes and lambs wore collars for four weeks at a time both years. Ewes' collars went through two design iterations. The first, which was used only for the first observation block of four weeks in 2021, was a waterproof rectangular plastic box containing the technology mounted onto black webbing with cable ties. The second, which was used for all other observations, consisted of elasticated polyester running belts with waterproof zipped pockets where the technology was kept. Lambs' collars were made of elasticated webbing with pockets containing the technology sewn onto the outside and Velcro® fasteners (Figure 4.1). All the materials to make ewe and lamb collars were purchased from online retailer Amazon (Seattle, USA). Sheep's necks were inspected at every fortnightly gathering event to ensure any injury caused by the collars was recorded. If any broken skin was present on the neck, the collar was removed at once and not put back on until the lesion had fully healed and the cause behind the injury had been addressed. The AX3 accelerometer recorded tri-axial coordinates at a rate of 12.5 times per second (12.5 Hz) and stored them on the device. These were downloaded using OmGui software (Open source, hosted by sourceforge.net) when the collars were removed. This created comma separated values (CSV) files which could be exported into Microsoft Excel for processing and analysis.

Figure 4.1. First ewe collar design (a), second running belt ewe collar design (b), lamb collar (c).

4.2.5 Data Analysis

Data analysis was carried out using R 4.3.0 (R Core Team, 2023) via R Studio (version 2023.03). Ewe and lamb data were analysed separately.

4.2.5.1 Collar Effects on Behaviour and Welfare

Welfare indicator and behaviour data from 2021 were combined with those from 2022 after being converted to the same scale and analysed as one dataset, as described in Chapter 3. To test the relationship between welfare scores and wearing collars, cumulative link mixed models (CLMM) [*ordinal* package (Christensen, 2022)] were used. Model fitness was verified by log-likelihood test in the *ordinal* package (Christensen, 2022). One model with each welfare score (lameness, dag, BCS etc.) as the response variable was created. The fixed effects tested were day of experiment (DOE), scan number (1,2,3,4), technology treatment group (Y,N), and year (2021,2022). The EID was included as the random effect.

Binomial GLMMs [*glmmTMB* package (Brooks et al., 2017)] were used to analyse strongyle and N*ematodirus* FEC (presence / absence (0,1)) due to the complex distribution of FEC data that made it impossible to achieve model fit when using it as a covariate. For strongyle eggs in ewes, presence (1) was described as a FEC of >200 epg, while absence (0) was any FEC equal to or below 199 epg. In lambs, presence of strongyle eggs was defined as any FEC other than 0 epg and absence was an FEC of 0 epg. To create binomial *Nematodirus* indicators for ewes and lambs, presence was defined as any FEC other than 0 epg and absence was an FEC of 0 epg. The model's fixed effects were as described for the CLMM.

Analysis of collar impact on behaviour was conducted using binomial GLMMs [*glmmTMB* package (Brooks et al., 2017)] where the response variable was the presence or absence (0,1) of a behaviour (e.g. grazing, standing, ruminating, etc.), fixed effects were lameness (0, 1, 2, 3, 4), presence of collar (Y,N), scan number (1, 2, 3, 4), while strongyle FEC (eggs/gram) and nematode FEC (eggs/gram) were included as covariates. Family ID (a number assigned to each replicate) with day of experiment (DOE) nested within it was included as a random effect. Behaviour sampling data and QBA data were analysed as described in 3.2.5.2 and 3.2.5.3, respectively. Model fit tests and pairwise comparisons were performed as described in 3.2.5.2.

4.2.5.2 Technology Validation

Accelerometer data were downloaded into CSV files and analysed in R. The raw datasets consisted of 3 columns of data corresponding to the three gyroscope axes: x, y, and z. The formula $\sqrt{x^2+y^2+z^2}$ was applied to all rows to create a motion index (MI) for each row of data (Fadel et al., 2020).

i. Timestamp validation

It was necessary to confirm that the timestamps on the AX3 data matched those from the in-person visual observations. Before collars were put onto animals on July 19th 2021, researchers put them in a bag and shook them vigorously for 10 seconds while being filmed with a GPS clock on the screen of a phone next to them. This allows for a clear demarcation in the AX3 data to be matched with the exact time of shaking. If any discrepancy existed, this difference could be applied to all AX3 data points to convert it to real time and be able to compare it with behavioural observations, which were timestamped using GPS time.

ii. Epoch selection

Graphs showing the mean MI (MMI) over the 20-minute observations were plotted for each animal. A colour key for each behaviour was created and the corresponding colour was layered onto the background of a graph to show behaviour and MMI in one object. These graphs were built for 1 sec, 30 sec, 1 min, 2 min and 5 min epochs. For reasons described in the results section, 1-minute epochs were selected to answer the research question, meaning the motion index reported was a mean motion index (MMI) from the 750 (12.5*60 seconds) records of MI in each minute. Additionally, mean, variance and range of x, y and z were put into 1-minute epoch columns alongside MMI to be assessed as potential activity indicators.

iii. Assigning an AX3 signature to behaviours

Following the creation of an MMI column in the R dataframe, CSV files were exported for each animal on the 4 observation days containing: date, time, and mean motion index (MMI) using a 1-minute epoch. In Excel, each row was manually matched with the behaviour from the behavioural observation datasheet. If multiple behaviours were performed in 1 minute, the one performed the longest portion of the minute was chosen. Another column was created describing the behaviour in each row as active or inactive (Table 4.2). This resulted in a dataframe where each row corresponded to a minute of an individual's behaviour with the associated MMI, mean value of x, y and z, variance of x, y and z, and range of x, y and z. These dataframes for all individuals across all observation days were combined for analysis. A range of statistical analyses was performed on this dataset to find the AX3 signature of each behaviour.

Table 4.2. Classification of observed behaviours into binary active/inactive categories for analysis.

Behaviour	Activity	Binomial Activity
Grazing	Active	1
Locomotion	Active	1
Lying	Inactive	0
Standing Rumination	Inactive	0
Standing Inactive	Inactive	0

Generalized Linear Mixed Model (GLMM)

Gaussian GLMMs were run using the package *glmmTMB* (Brooks et al., 2017) to investigate the relationships between the various AX3 outputs and the behaviours, while accounting for individual variation. The response variable was MMI while behaviour (factor with 5 levels: grazing, locomotion, lying, standing rumination, standing inactive), and type (ewe, lamb) were the fixed effects, time (seconds since first observation) scaled to a mean of 0 and standard deviation of 1 was a covariate and EID was the random effect. Separate models with the various AX3 outputs (mean of x, variance of x, range of x, etc.) as the response variables were run with the same explanatory variables. When the above GLMMs did not run due to lack of variation in the data, binomial GLMMs were run with activity (0-inactive, 1-active) as the response variable, where the categorisation of behaviours was based on Table 4.2. Type (ewe, lamb) was a fixed effect, time (seconds since first observation) scaled to a mean of 0 and standard deviation of 1 and MMI were covariates and EID was the random effect. A second binomial random slope model was run with the same fixed effects as the first binomial model but EID was nested within MMI as a random effect to account for individual variations in behaviour expression. These binomial analyses were run on the 1-minute epoch dataset and the 1-second epoch dataset.

Generalized Additive Mixed Models (GAMM)

Binomial GAMMs were run using the package *gamm4* (Wood & Scheipl, 2014) to examine the non-linear relationship between binomial activity levels (active-1, inactive-0) and MMI. A GAMM is a GLMM in which the linear predictor depends on unknown smooth functions assigned to the covariates (called smooths) (Wood & Scheipl, 2014). This allows for more realistic representations of non-linear relationships, such as the one that most likely exists between time of day and sheep behaviour. Smooths were applied to the covariates time and MMI, while type (ewe, lamb) acted as a factor and EID was the random effect. The parameter estimates of smooth terms were plotted using the package *ggplot2* to visualise the non-linear relationship between activity and MMI.

K-means cluster analysis

To group data points together based on unknown parameters, K-means cluster analysis was applied using the *cluster* package (Maechler, 2018). K-means is a type of unsupervised machine learning algorithm that groups data points into *k* groups (i.e. clusters), where *k* is the number of groupings selected by the analyst. Once grouped, the sheep in each cluster and the behaviours they performed were manually examined for patterns. The appropriate number of clusters was determined by combining 3 methods. First, the Elbow method, which graphs the total within-cluster variation (or within sum of squares) of each possible number of clusters, was applied. Secondly, the Silhouette Method measures the quality of a clustering by determining how similar a data point is within-cluster compared to other clusters. A high average silhouette width indicates a good clustering. Finally, the Gap Statistic Method was applied, which compares the total intra-cluster variation with their expected values in a distribution where there is no clustering (Tibshirani et al., 2001). All 3 methods were used to strongly support the decision on the final number of clusters. Partition percentage describes the quality of a k-means grouping, with higher percentages indicating a better partition. They are calculated by dividing the between cluster sum-of-squares by the within cluster total sum-of-squares and multiplying by 100 to obtain a percentage. However, partition percentages always increase as numbers of clusters increase, so in the present analysis, partition percentages of cluster numbers suggested by the 3 methods were compared to attempt to select the highest partition percentage without overly or unnecessarily subdividing the data. Silhouette plots, which illustrate measures of how close each point in one cluster is to points in the neighbouring clusters, were then produced to finalise how many clusters led to an optimal division of observations. These plots provide a silhouette width for each data point, which is a value from -1 to 1. Values near +1 indicate that the data point is far away from neighbouring clusters and that the grouping is therefore robust. A value of 0 indicates that the data point is on or very close to the decision boundary between two neighbouring clusters and negative values indicate that the data point may have been assigned to the wrong cluster.

A Principal Component Analysis (PCA) was then run on the clusters to visualise their relationship to each other using the *factoextra* package (Kassambra & Mundt, 2020). The observations belonging to each cluster were printed using the base R *print* function and cluster ID was added as a column to the dataframe. Plots were then created using the base R *plot* function to compare the mean, variance and range of x, y, and z across clusters.

Hidden Markov Models (HMM)

HMM are machine learning models that are trained on datasets where observations are attributed to known "states," and then applied on similar observations where the states are unknown with the aim of discovering them. In the present study, the model was trained on the validation dataset of a single lamb (ID: 13876) at the 12.5Hz epoch where the observed behaviour at that time was the known state. Unlike the other methods, this was tested on the 12.5 Hz data in an attempt to capture as much variation as possible. The R packages *HMM* (Himmelmann, 2022) and *markovchain* (Spedicato, 2017) were used. A transition probability matrix for behaviours was created using the *markovchainFit* function. In theory, the trained models could then be applied to all the AX3 outputs and attribute a behaviour to each observation. Support was provided by machine learning expert Dr Juan Morales from the University of Glasgow.

4.3 Results

4.3.1 Collar Effects on Welfare

4.3.1.1 Scan Sampling

Lambs wearing collars were no more or less likely to graze (p=0.568), walk (p=0.248), lie (p=0.908), ruminate (p=0.352) or stand (p=0.287) than control lambs (Figure 4.2b). In ewes, wearing a collar (prob=0.264, SE=0.034) resulted in a lower likelihood of performing rumination behaviour compared to not wearing one (prob=0.299, SE=0.037) (OR=1.190, SE=0.100, 95% CI=1.010-1.400, p=0.040). Collars did not affect ewe grazing (p=0.301), locomotion (p=0.287), or lying (p=0.060) (Figure 4.2a).

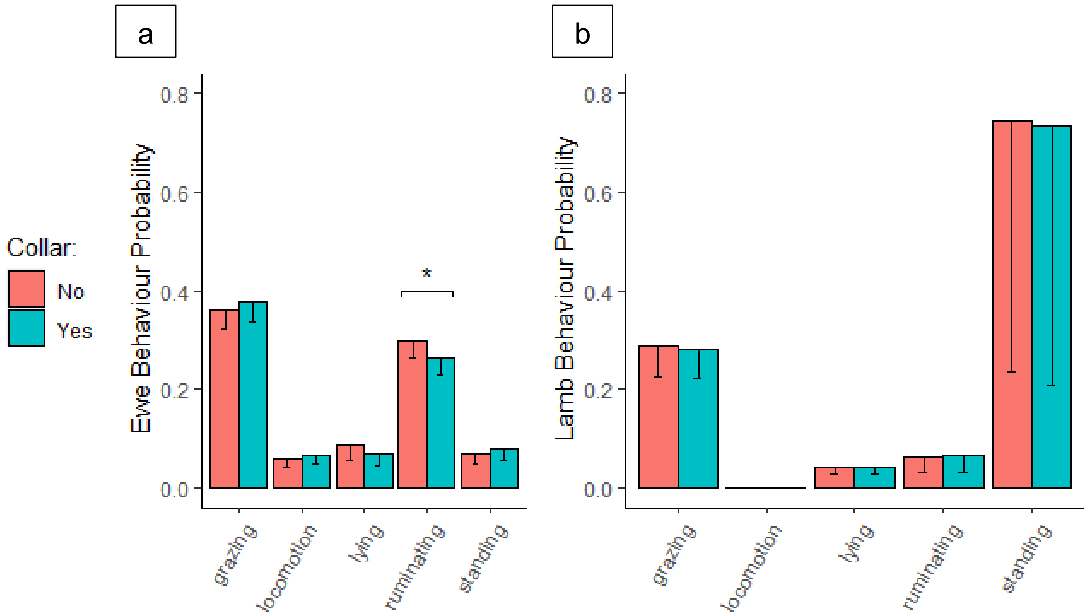

Figure 4.2. Ewe (a) and lamb (b) probabilities of performing behaviours with and without PLF collars with only lower error bars shown to preserve column scale.

4.3.1.2 Behaviour Sampling

Collar treatment group did not have a significant impact on play and sucking behaviours in lambs observed through behaviour sampling in 2021. There were no significant differences in the number of play bouts (p=0.657) or duration of play (p=0.498) between lambs with collars and those without. The models examining average duration of play bouts and count of sucking bouts could not be fitted due to very low variation in the data. However, there were no significant differences between lambs with and without collars for total duration of sucking bouts (p=0.154) or average duration of sucking bouts (p=0.187). No behaviour sampling was conducted in 2022.

4.3.1.3 Qualitative Behaviour Assessment

i. Data from 2021

Dimension interpretation from the PCA for both years is described in Chapter 3. Collar treatment group did not affect lamb loadings on PC1 (arousal)($p=0.260$), PC2 (valence)($p=0.822$), or PC3 (sociality)($p=0.374$).

ii. Data from 2022

Lamb loadings on PC1 (arousal) were significantly higher in lambs without collars (5.63±0.30) than in lambs with collars (4.30±0.31)($t=3.029$, 95% CI=0.46-2.20, $p=0.003$) (Figure 4.3). Lamb loadings on PC2 (valence) were not affected by wearing collars ($p=0.481$). Ewes' loadings on PC1 ($p=0.992$) and PC2 ($p=0.967$) were not affected by wearing collars.

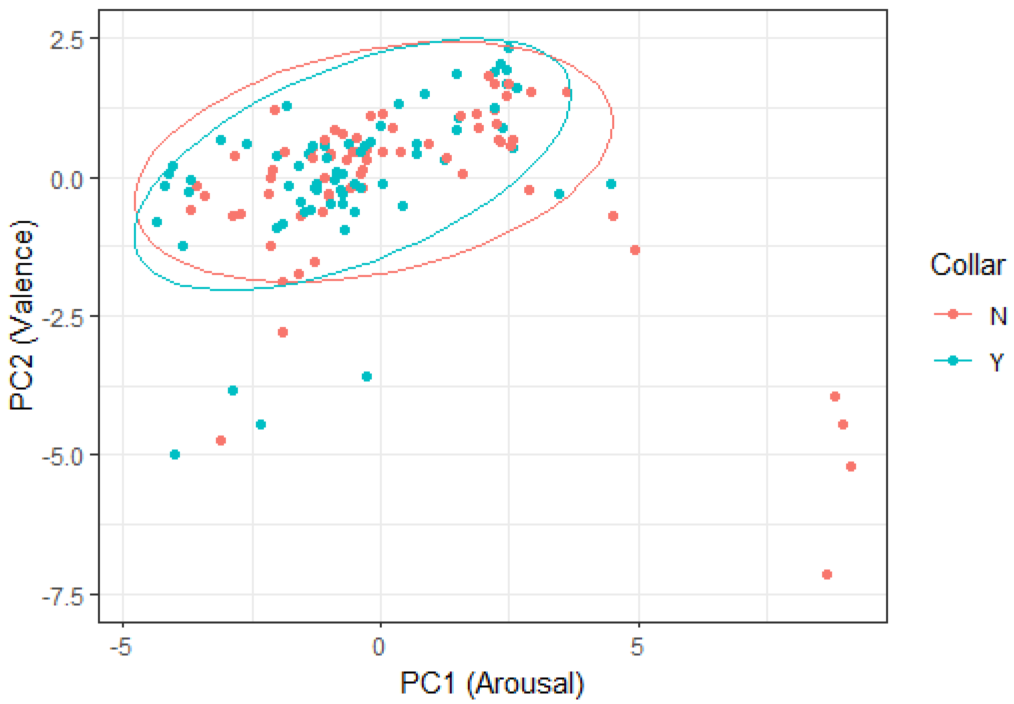

*Figure 4.3. Principal Component Analysis loading plots on PC1 (arousal) and PC2 (valence) (transformed by *-1 for ease of graphing) for lambs without collars (pink, N) and with collars (blue, Y) in 2022.*

4.3.1.4 Welfare Indicators

Collars did not affect ewe welfare indicators (Table 4.3) as assessed by lameness score (p=0.924), mastitis score (p=0.636), BCS (p=0.170), or binomial *Nematodirus* FEC (p=0.852). Most lamb welfare indicators were similarly unaffected by collars. No significant differences in lameness score (p=0.416), dag score (p=0.121), and binomial *Nematodirus* FEC (p=0.733) existed between lambs wearing collars and control lambs (Table 4.3). However, collars had a significant association with ewe and lamb binomial strongyle FEC (p=0.013 and p<0.001 respectively), suggesting that the collars may have affected grazing behaviour and limited access to larvae on grass (Table 4.3).

Table 4.3. Pairwise comparisons of means of welfare indicators for lambs and ewes. Rows marked with an asterisk report probability rather than mean in the Mean/Probability column because they are the result of a binomial GLMM.

Welfare Indicator	Collar	Mean/ Probability*	SE	p-value of pairwise comparison
Lambs				
Lameness score	N	1.02	0.004	0.416
	Y	1.02	0.003	
Dag score	N	1.56	0.139	0.149
	Y	1.88	0.185	
Binomial *Nematodirus* FEC*	N	0.624	0.039	0.426
	Y	0.668	0.039	
Binomial strongyle FEC*	N	0.980	0.011	<0.001***
	Y	0.969	0.017	
Ewes				
Lameness score	N	1.01	0.004	0.924
	Y	1.01	0.004	
Mastitis score	N	1.60	0.124	0.636
	Y	1.69	0.139	
Body condition score	N	2.21	0.080	0.170
	Y	2.07	0.076	
Binomial *Nematodirus* FEC*	N	0.083	0.033	0.853
	Y	0.078	0.031	
Binomial strongyle FEC*	N	0.181	0.039	0.013**
	Y	0.079	0.026	

4.3.2 Accelerometer Validation

4.3.2.1 Timestamp validation

A video from July 19th, 2021 at 10:04 showing a researcher shaking the collars containing the AX3 from 10:04:50 to 10:05:00 was recorded. Graphs of MI from the collars show a significant spike during those 10 seconds, meaning the AX3's timestamps corresponded to real time and did not need to be adjusted for analysis (Figure 4.4).

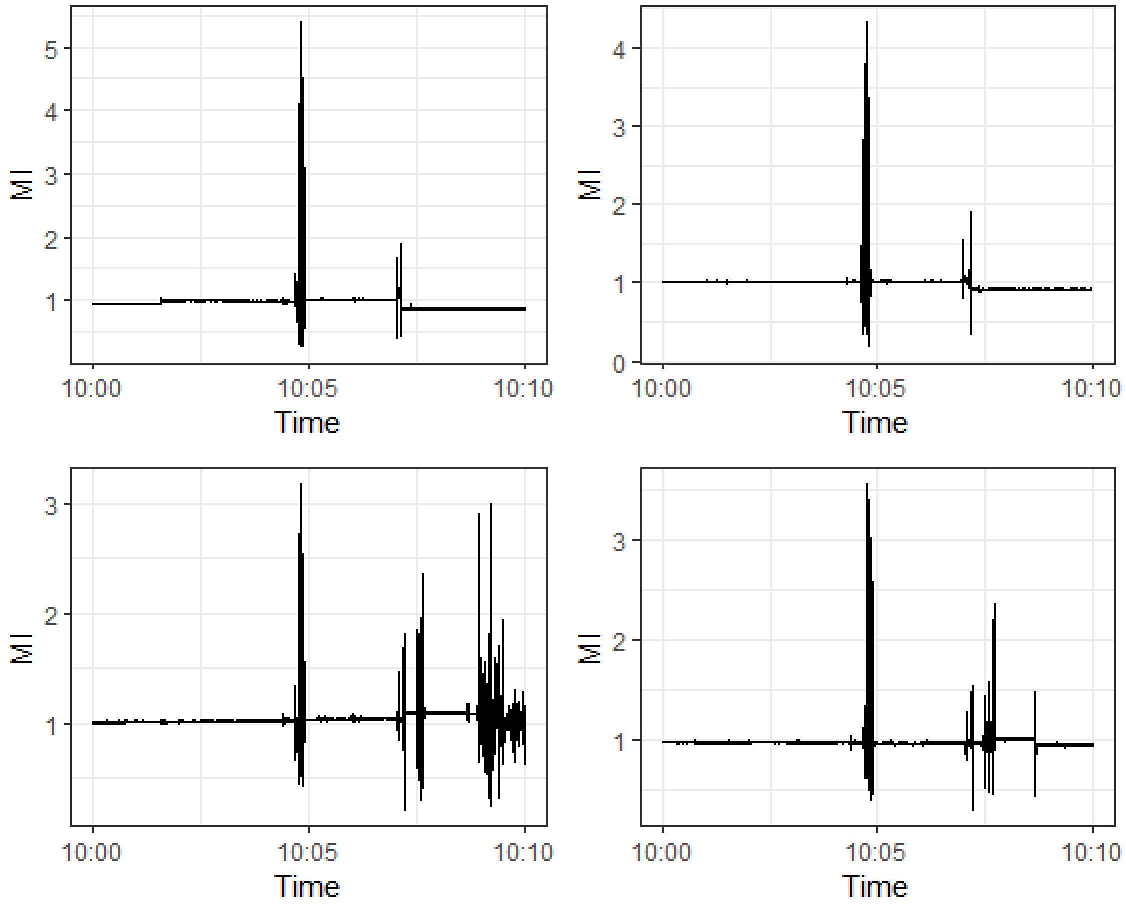

Figure 4.4. Graphs of motion index (MI) over time from an example of four accelerometers that were shaken from 10:04:50 to 10:05:00 on July 19th, 2021, to validate the devices' timestamps.

4.3.2.2. Epoch Selection

Graphs depicting 1 and 30 second epochs were overly noisy and a clear delineation between behaviours was not visible (Figure 4.5a, 4.5b). Using 5-minute epochs, the MMI line was smoothed to an extent where only major changes were visible (Figure 4.5d). One-minute epochs allowed for presumed within-behaviour changes to be seen, e.g. head bobbing while grazing and walking, as well as showing presumed changes in activity, for example between standing and grazing (Figure 4.5c).

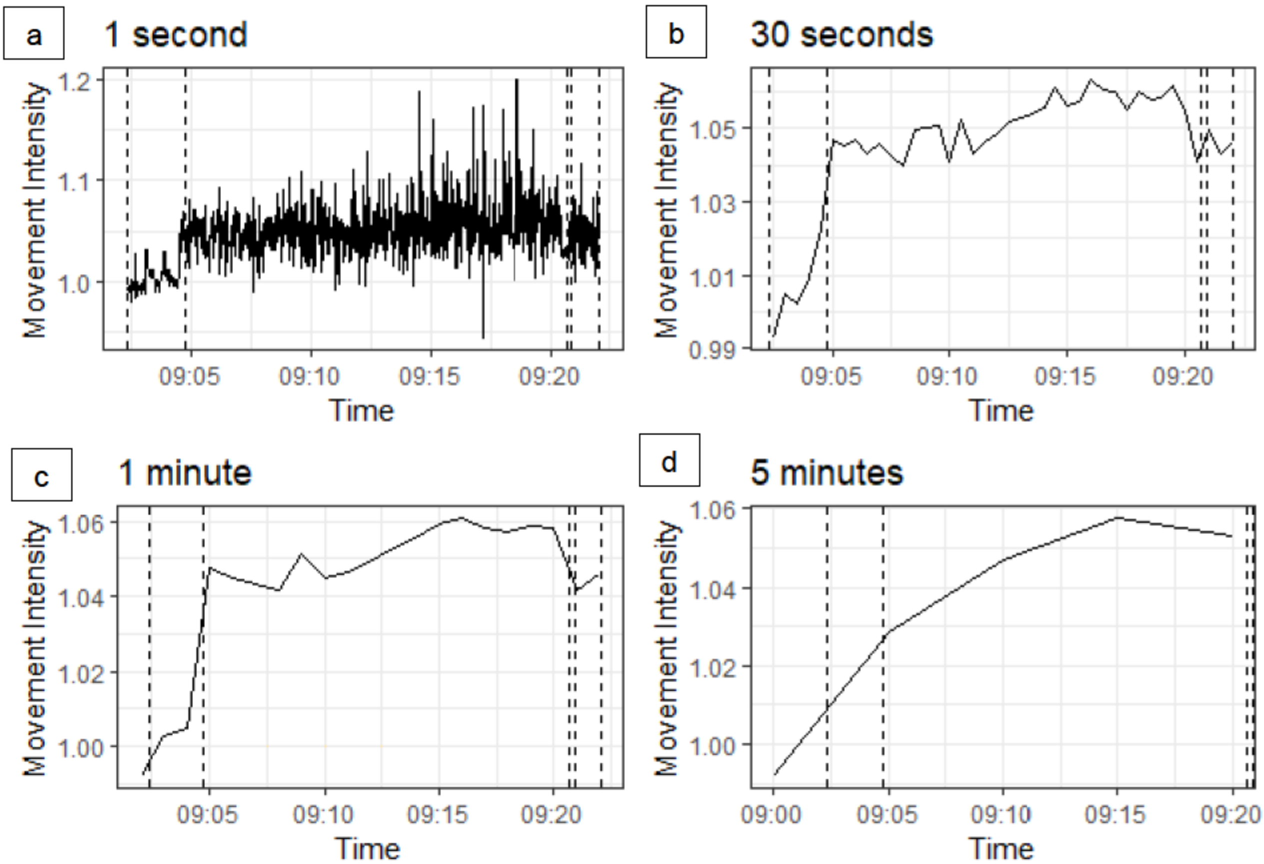

Figure 4.5. Twenty minutes of data from the same accelerometer shown at a 1-second (a), 30-second (b), 1-minute (c) and 5-minute (d) sampling rate, or epoch. The dotted lines mark a change in observed behaviour.

4.3.2.3 Assigning MMI to behaviour and activity

When behaviours were assigned to each 1-minute epoch from the validation observation period as described in 4.2.5.2, the most common behaviour was grazing, and the least common was lying (Table 4.4).

Table 4.4. Counts of how many times each behaviour was recorded and how many animals were observed to have performed each behaviour at least once across the validation observations.

Behaviour	Count of behaviour records	Number of animals who performed behaviour
Grazing	139	7
Locomotion	7	3
Standing	16	4
Standing Rumination	4	3
Lying	1	1

i. Generalise Linear Mixed Models (GLMM)

Gaussian GLMMs could not be fitted due to very low levels of variation in the behaviourial data. Even after transformation (sqrt(max(MMI+1) - MMI)) and scaling of MMI (for a mean of 0 and standard deviation of 1), the model fit remained poor (Figure 4.6a). The Kolmogorov-Smirnov (KS) test result presented in the QQ-plot by the *DHARMa* package was highly significant, indicating that the residuals were not normally distributed and the quantile regression detected a significant deviation from uniformity in the residuals in y-direction (Figure 4.6b).

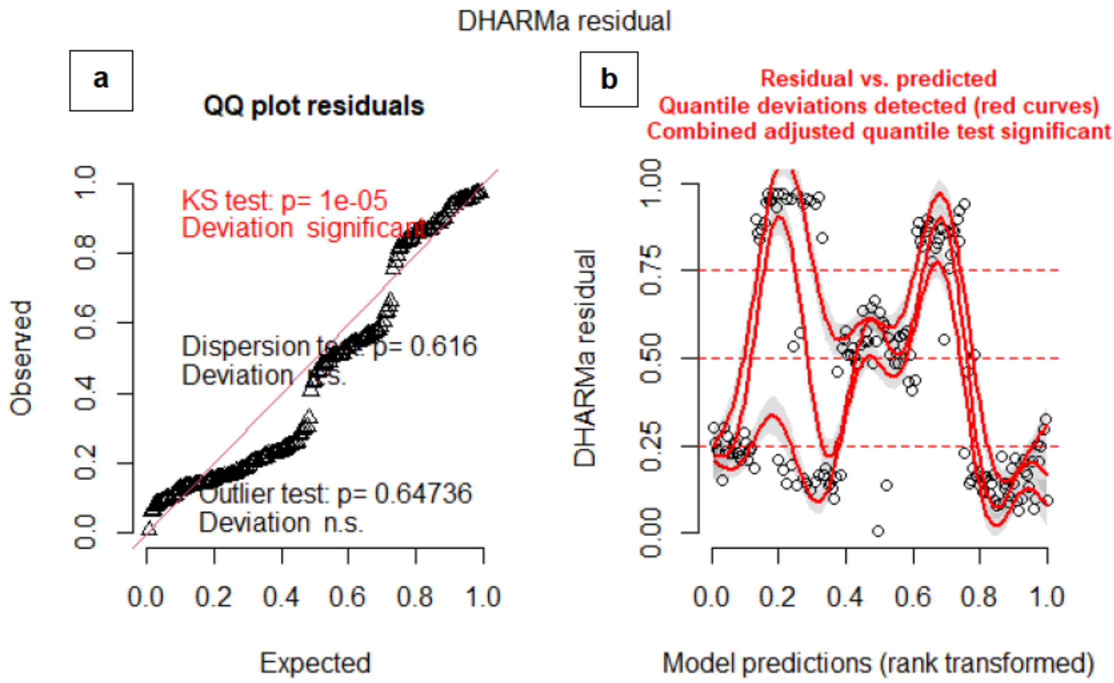

Figure 4.6. Residuals diagnostics plots for Gaussian GLMMs where MMI (scaled and transformed) was the response variable. QQ-plot (a) shows that the distribution of residuals significantly deviates from the expected normal distribution. The plot of residuals over predicted values (b) shows significant deviations from y-direction uniformity in the residuals.

Visually, there was no difference in the MMI of behaviours recorded in the 1-minute epoch data (Figure 4.7). The same was true of mean values of x, y and z, variances of x, y and z and ranges of x, y and z (Figure 4.8).

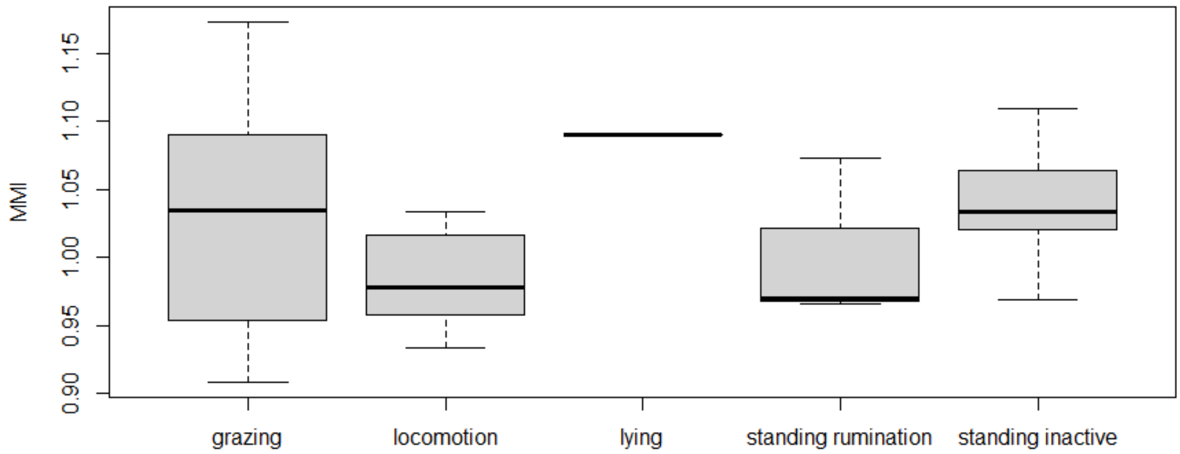

Figure 4.7. Boxplot of mean motion index (MMI) of each behaviour performed by ewes and lambs during the validation observations analysed with a 1-minute epoch, showing the median, lower quartile (Q1), upper quartile (Q3), minimum and maximum for each behaviour.

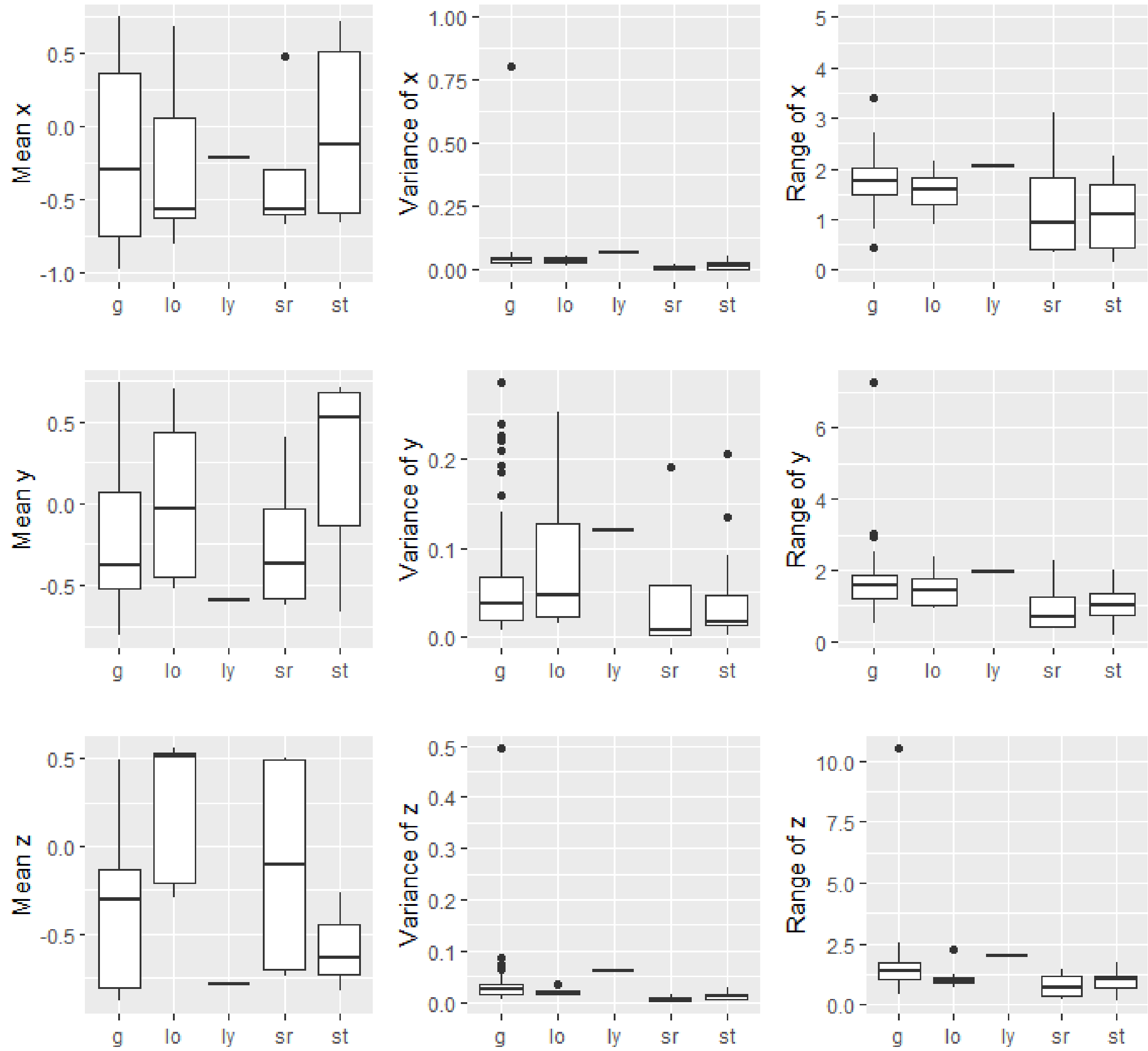

Figure 4.8. Boxplots of the mean, variance and range of x, y and z for each behaviour performed by ewes and lambs during the validation observations analysed with a 1-minute epoch, where g=grazing, lo=locomotion, ly=lying, sr=standing ruminating, st=standing inactive.

When behaviours were grouped into active (grazing, locomotion) and inactive (lying, standing rumination and standing inactive) categories, no difference could visually be identified between them (Figure 4.9). Equally, binomial modelling revealed no effect of time, MMI or type on behaviour. In fact, the null GLMM where MMI was not included (AIC=133.02) had similar but slightly better fit than the model with MMI as a covariate (AIC=134.72), meaning there is likely no linear relationship between MMI and the binomial active/inactive variable in this dataset.

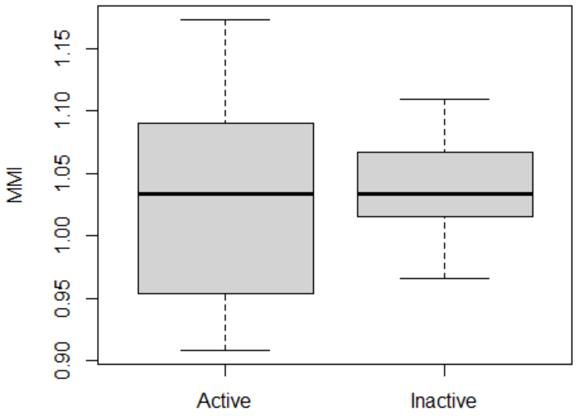

Figure 4.9. Boxplot of mean motion index (MMI) of active and inactive categories of behaviours performed by ewes and lambs during the validation observations analysed with a 1-minute epoch, showing the median, lower quartile (Q1), upper quartile (Q3), minimum and maximum.

The binomial GLMMs using the 1-second epoch dataset suggested that the random slope model (AIC=12008.79) had a better fit than the binomial model with only EID as a random effect (AIC=14587.45). In the random slope model, MMI significantly increased as activity increased (est=28.18, SE=8.80, z=3.20, p=0.001). However, this positive relationship did not apply to every individual, as shown in Figure 4.10.

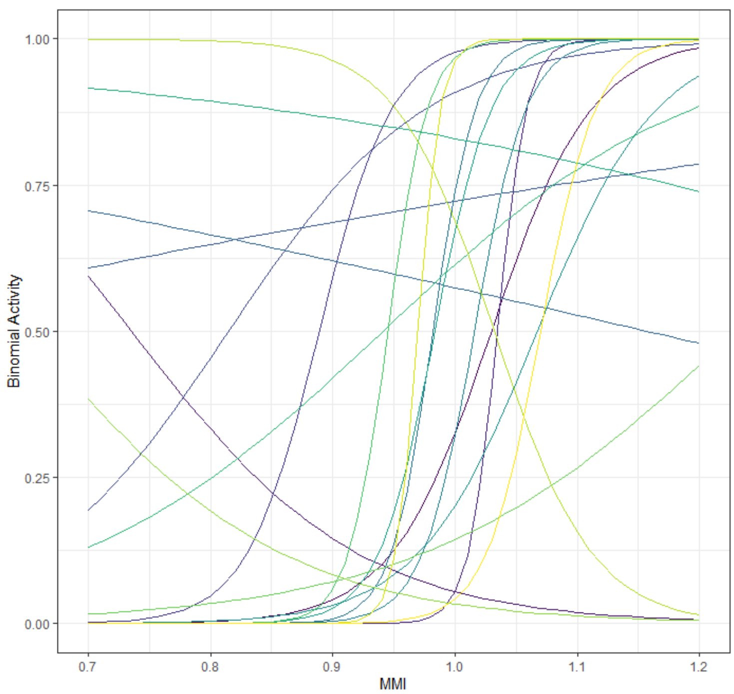

Figure 4.10. Random slope binomial GLMM prediction of relationship between binary activity levels and MMI, where each line represents one individual sheep (ewes and lambs are included) in the validation study.

ii. Generalise Additive Mixed Models (GAMM)

MMI and time with their respective smooths applied had significant positive relationships with behaviour. As activity increased, MMI increased (est=10.31, SE=3.34, z=3.09, p=0.002), as did time (est=7.73, SE=1.22, z=6.34, p<0.001).

Plotting the predicted relationship between MMI and activity resulting from the GAMM shows a plateau once MMI reaches approximately 1.6 and much variation in possible activity level around MMI 1.0 (Figure 4.11).

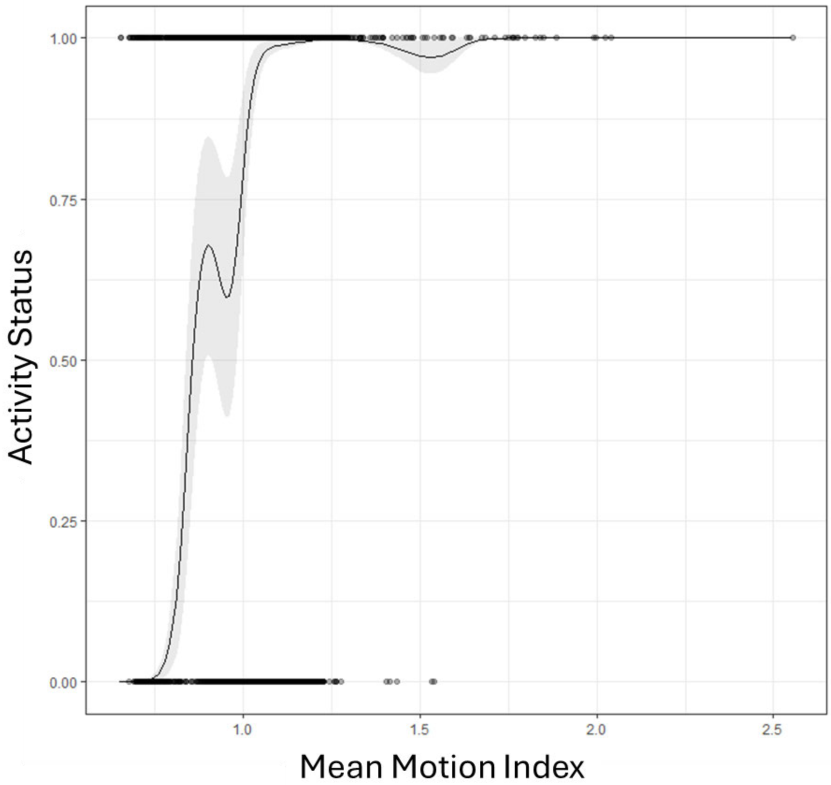

Figure 4.11. General Additive Mixed Model prediction of Mean Motion Index based on activity levels, where 0 is inactive and 1 is active.

The GAMM allows us to conclude that it is likely that higher MMI's indicated higher levels of activity, but based on these results, MMI cannot be used to distinguish between behaviours such as grazing, locomotion or standing.

iii. K-means clustering

The Elbow method suggested between four and seven clusters for the dataset (Figure 4.12). The silhouette method recommended eight clusters (Figure 4.12). The gap statistics method suggested one cluster, meaning the data should not be clustered at all (Figure 4.12). Since these results from the 3 equally rigorous methods were conflicting, scenarios with all the suggested cluster numbers aside from 1 were tested.

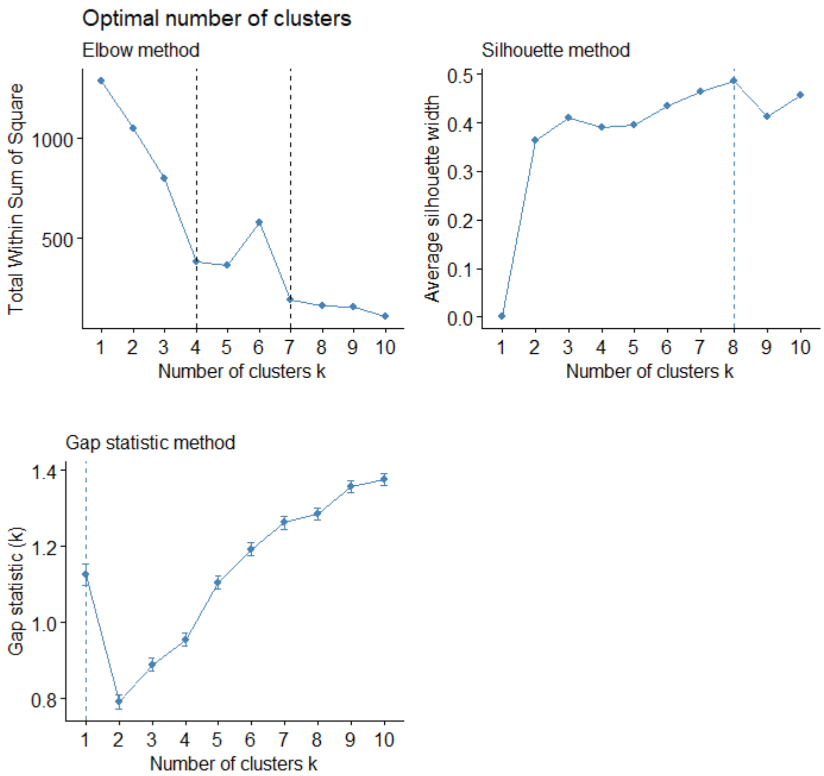

Figure 4.12. Optimal numbers of clusters for k-mean clustering shown by dotted vertical lines according to three methods.

As described in 4.2.5.2.iii, high partition percentages reflect a better clustering of data points, but it must be underlined that the percentage will always increase as cluster number increases. Partition percentage with four clusters was 70%, while at seven clusters it was 85%. With eight clusters, the partition percentage was 87%. Silhouette plots with each possible number of clusters revealed that eight clusters was likely too many because clusters 4 and 5 only had one observation each (Figure 4.13a). Additionally, observations in cluster 1 had a negative silhouette width meaning they had not been classed in the appropriate cluster (Figure 4.13a). The average silhouette width of 0.47 was relatively low (Figure 4.13a). Four clusters had a similarly low average silhouette width and one cluster containing only one observation (Figure 4.13b). The silhouette plot with seven clusters had the highest average silhouette width, although still relatively low at 0.49 (Figure 4.13c). Some observations were negative and again, one cluster only contained one observation (Figure 4.13c). Given no cluster number choices resulted in ideal silhouette plots, seven clusters were chosen for analysis given its higher average silhouette width of 0.49 (Figure 4.13c).

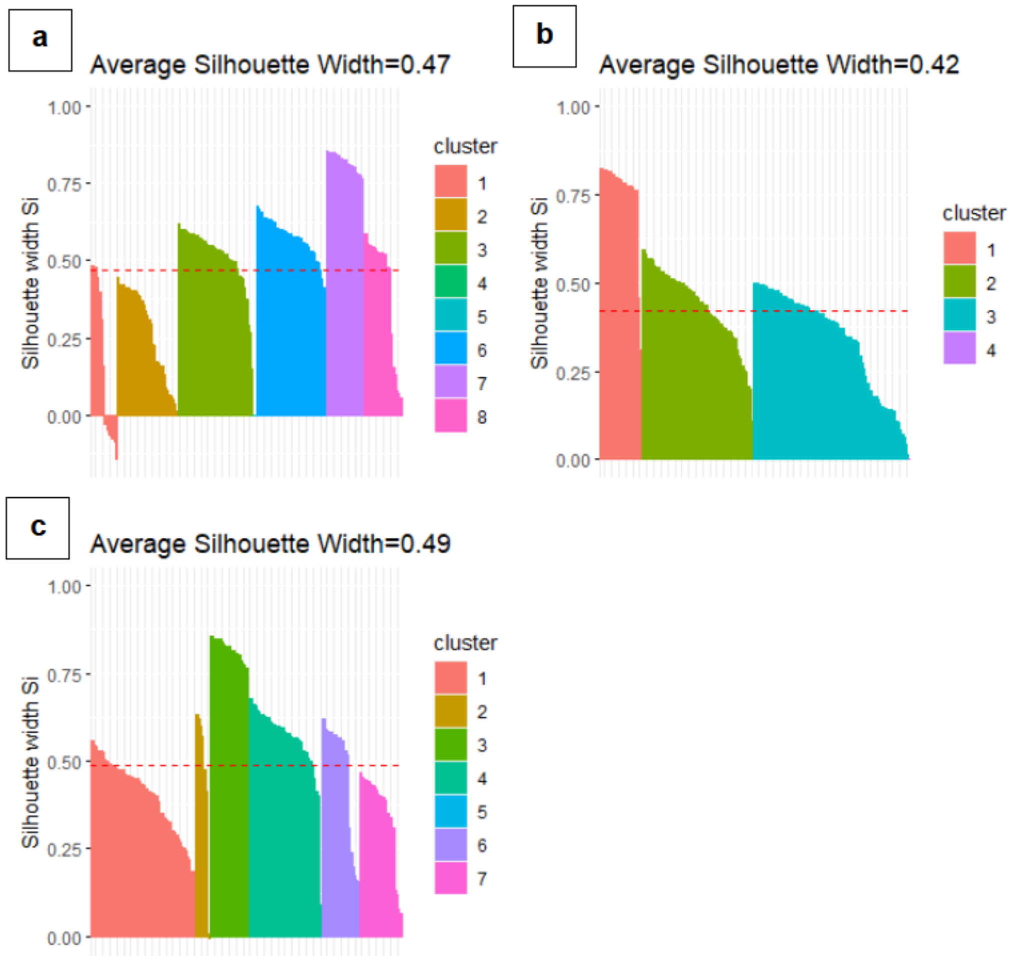

Figure 4.13. Silhouette plots of different cluster number possibilities: eight (a), four (b) and seven (c).

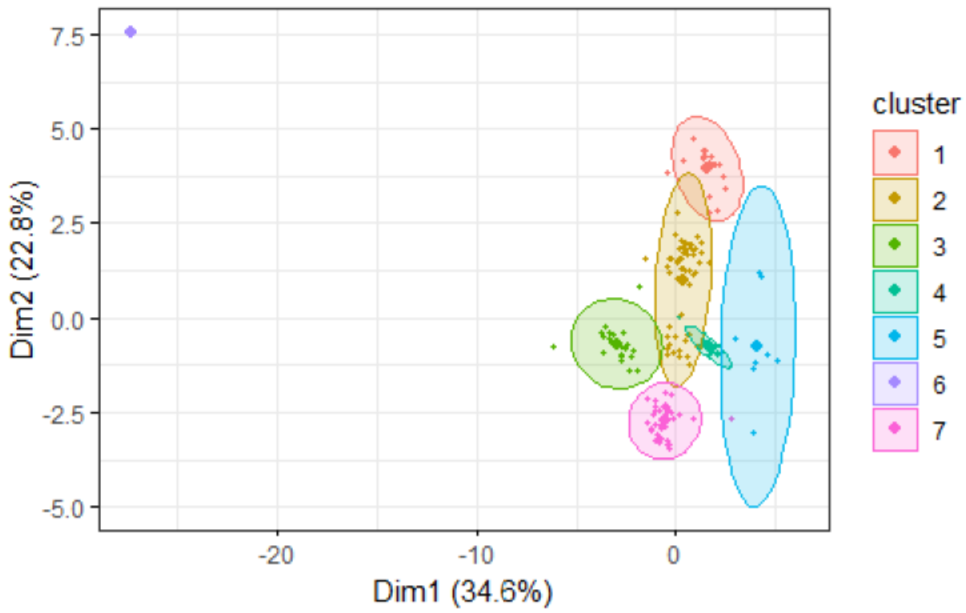

Figure 4.14. Cluster plot of a principal component analysis of seven clusters of accelerometer data determined by k-means clustering, where each dot is one observation except for the largest dot in the centre of each cluster, which represents the cluster centroid.

The PCA cluster plot in Figure 4.14 shows that cluster 7 contained a single observation which was an outlier. The other 6 clusters show very little variation along PCA Dimension 1, and some variation along dimension 2 despite many clusters overlapping (Figure 4.14). At this stage it was concluded that the large amount of overlap across clusters likely meant there was little variation in the dataset. A new column was added to the dataframe assigning a cluster to each observation. Graphs of AX3 variables separated by clusters were generated to visually identify which variables had the most differences across clusters (Figure 4.15).

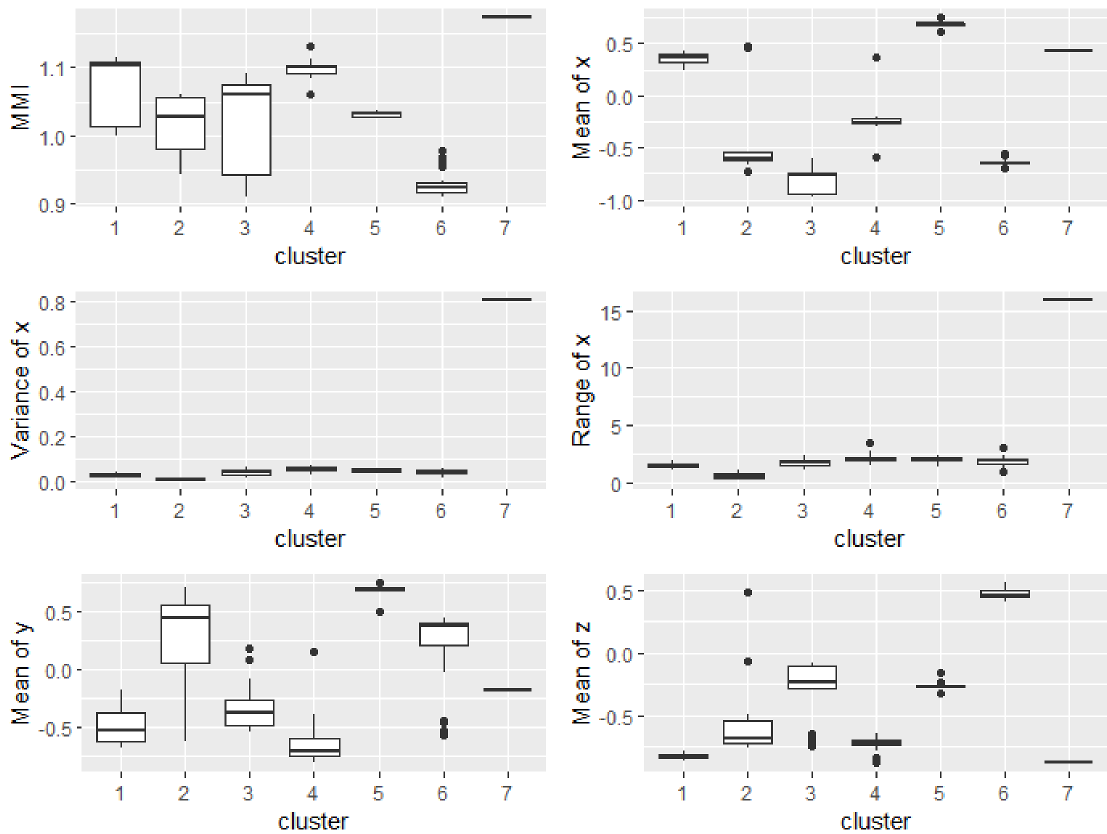

Figure 4.15. Boxplots of AX3 variables across the seven clusters identified by the k-means method.

MMI, mean of x, y, and z were identified as potentially useful variables to describe the differences between clusters due to their relatively high levels of variation. These were modelled using Gaussian GLMMs after transformation (1/Y+2) to normalise them. The only variable where model fit was acceptable was mean of y, likely because of its higher within-cluster variation. Pairwise comparison showed that the clusters were mostly significantly different from each other (Table 4.5).

Table 4.5. P-values of pairwise comparisons of mean y between clusters.

	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7
Cluster 1	<0.001	0.002	<0.001	<0.001	<0.001	0.600
Cluster 2		<0.001	<0.001	0.010	0.987	0.839
Cluster 3			<0.001	<0.001	<0.001	0.972
Cluster 4				<0.001	<0.001	0.060
Cluster 5					<0.001	0.155
Cluster 6						0.935

Through filtering, the type (ewe or lamb) of sheep and the activity performed in each cluster was examined. Cluster 1 (C1) contained observations of lambs grazing and three observations of lambs standing inactive. C2 contained records of lambs and one ewe standing inactive, standing ruminating and grazing. C3 had observations of ewes mostly grazing, but two were standing inactive, one was performing locomotion, and one was standing ruminating. C4 contained lambs and one ewe that were all grazing, except for one lamb lying. C5 contained observations for one single ewe performing standing inactive, locomotion and grazing. C6 had observations from one single lamb performing standing ruminating, locomotion and grazing. C7 only contained one observation of a lamb grazing.

If the exceptions in each cluster are crudely ignored, it could broadly be understood that C1 represents lambs grazing, C2 is lambs standing, C3 is ewes grazing and C4 is lambs grazing again. C5 and C6 seem to be the result of significant individual variation that caused one lamb and one ewe to be alone in their clusters, while C7 is most likely an outlier as it contains a single observation (Table 4.6). Interpretation accuracy was calculated as a percentage of observations in the cluster that match the behaviour the cluster is presumed to represent (Table 4.6). For example, 92% of observations grouped by the k-means into cluster 1 were of lambs grazing.

Table 4.6. Descriptive statistics of the mean y value of seven clusters and their possible interpretation within the validation dataset.

Cluster	Mean y	SE	Possible Interpretation	Interpretation accuracy %
1	- 0.487	0.024	Lambs grazing	92
2	0.270	0.103	Lambs standing	21
3	- 0.346	0.023	Ewes grazing	92
4	-0.646	0.043	Lambs grazing	91
5	0.685	0.010	Ewe 322 only, many behaviours	NA
6	0.186	0.082	Lamb 13859 only, many behaviours	NA
7	- 0.176	NA	Lamb 14253 grazing	NA

iv. Hidden Markov Models (HMM)

The transition probability matrix revealed that all behaviours were most likely to happen following a display of the same behaviour (Table 4.7). On further exploration of the validation dataset, Dr. Juan Morales advised that HMM was not suitable as the AX3 variables, including MMI, displayed too little variation across behaviours.

Table 4.7. Transition probability matrix of behaviours performed by ewes and lambs according to the validation observations showing the probability of performing one behaviour immediately after another.

	Bleating	Grazing	Locomotion	Lying ruminating	Playing	Standing inactive
Bleating	0.923	0.0769	0	0	0	0
Grazing	0.00003	0.999	0.00003	0.00003	0.00003	0.00003
Locomotion	0	0.008	0.992	0	0	0
Lying ruminating	0	0	0	1	0	0
Playing	0	0.00004	0	0	0.999	0
Standing inactive	0	0.037	0	0	0	0.963

4.4 Discussion

Wearing collars containing technology appeared to impact ewe ruminating behaviour, ewes and lambs without collars were more likely to have strongyle eggs in their faecal samples and lamb loadings on the arousal dimension of the PCA were higher in lambs without collars compared to lambs wearing collars. The association with binomial FEC may be due to the collars impacting grazing behaviour and therefore exposure to parasite larvae on grass. All GLMMs, GAMMs, and HMMs were unsuccessful in differentiating between behaviours performed by sheep wearing collars containing AX3 accelerometers. However, k-means clustering, a type of unlabelled machine-learning, was able to identify grazing and standing in lambs and ewes based on the AX3 mean y value with high interpretation accuracy.

The initial test to examine whether wearing collars affected sheep behaviour is a crucial step in the development of any PLF tools destined to be worn by animals. Not only could the tool fail to perform its function in a precise manner if it did affect animal behaviour, but it could have a direct negative impact on welfare. This risk was brought up as early as 2005 in a paper on remote sensing of wildlife (Ropert-Coudert & Wilson, 2005). The authors considered the deleterious effects that large, unadapted wearable sensors can have on wild animals (Ropert-Coudert & Wilson, 2005). Similarly, farm animals' comfort must be taken into account before they are equipped with technology to avoid sores developing or getting caught on fences, for example (Herlin et al., 2021). The present study's findings suggest that ewes may alter their rumination behaviour when wearing collars, though the effect size was small. Previous studies have reported a lack of difference in behaviour in cattle and sheep with and without collars, though methods for analysing this vary (Barwick et al., 2018; Manning et al., 2017). It is possible that the difference in behaviour and QBA results found in this study is caused by discomfort around the neck brought on by the collars. This hypothesis could also explain the relationship identified whereby ewes and lambs without collars had an increased likelihood of strongyle eggs in faecal samples. This may be due to collars causing discomfort and limiting grazing behaviour, thereby decreasing exposure to strongyle larvae on grass, although no collar effects on grazing behaviour were identified in the models. This finding is further complicated by the fact that it is based on a binomial measure of strongyle infection, where presence was described as a FEC of >200 epg and absence was any FEC equal to or below 199 epg for ewes. In lambs, presence of strongyle eggs was defined as FEC above 0 epg and absence was an FEC of 0 epg. Parasites are most often overdispersed in sheep flocks, meaning few individuals carry most of the worm burden (Sréter et al., 1994). This is reflected by the negative binomial distribution of faecal egg count data in sheep (Sebatjane et al., 2019; Sréter et al., 1994). However, when sample size is small, it can be hard to verify this negative binomial distribution (Vidyashankar et al., 2012). This was the case in the present study, so FEC was transformed into a binomial factor to allow for model convergence. While it remains a viable indicator of infection, as used in Vidyashankar *et al.* (2012), results based on binomial FEC must be interpreted with caution. Other behavioural indicators recorded, such as lamb play behaviour, a known positive welfare indicator (Held & Špinka, 2011; Mellor, 2012), were unaffected by the collars. However, that wearing collars was associated with lamb arousal loadings in QBA may support the idea that they are having an effect on lamb mental state, possibly leading them to behave in a less active way. To clarify any associations between wearing collars and behaviour, and to draw robust conclusions on the welfare impact of sheep wearing collars containing technology, further research is needed. The impacts of wearable PLF tools on animal behaviour and their weights or dimensions are rarely detailed in the literature. Publishing these specifications could guide and support PLF development. Future studies could observe the same animals before, during and after wearing collars, as Manning *et al.* (2017) did, to ensure this result is not due to individual differences in behaviour and to avoid having to use group means of FEC.

Although the time stamp validation was successful, there is a phenomenon called time drift that entails a digital tool's clock "drifting" in relation to real time, causing observations to be recorded as having occurred at incorrect times. Drift is inherent to all digital tools, and results from random and systematic errors in oscillators (Guggenberger et al., 2015). For the present study, it is impossible to know if this occurred, because the timestamp validation test was not repeated after the first time. In future research, regular tests would ensure no time drift has occurred.

Epoch selection varies widely across devices and studies. For example, a machine-learning algorithm was successfully applied to identify ewe behaviour using 10 and 30 second epochs for accelerometer data (Fogarty et al., 2020) and the use of IceQube and IceTag (IceRobotics Ltd, Edinburgh, UK) sensors were validated to record postures and number of steps in lambs with a 15 minute epoch (Högberg et al., 2020). Our devices were set to 12.5 Hz, but I chose to filter the raw data using a 1-minute epoch. The 1-minute epochs were chosen because the sheep behaviours often occur for more than 1 minute, for example grazing, and I was limited by my computers' insufficient power, which was unable to quickly process the large and high-definition raw data.

GLMMs were first considered to study MMI and other AX3 outputs for their simplicity when studying repeated measures from the same animals. However, the difficulty in fitting any models made it apparent that the validation data was poorly suited to this model type. This was likely caused by the lack of variation in MMI, which mostly hovered between -1 and 1, and the fact that the relationship between MMI, time and behaviour is unlikely to be linear. It is difficult to imagine that a sheep's MMI (or activity levels) would increase in a truly linear fashion over time or across behaviours. In fact, there is evidence that sheep behaviour actually follows fractal-like patterns (Burgunder et al., 2018). It was for this reason that GAMMs were considered, since they do not assume a linear relationship between variables. With the R package *gamm4*, non-parametric curves were fit to the continuous variables of interest. However, the models still faced the problem of low variation in the data, as shown by the large amount of uncertainty in the model predictions around an MMI of 1, as seen graphically in Figure 4.11.

The GAMM did enable us to conclude that for most individuals a higher MMI resulted from higher activity levels. However, the opposite was true for certain individual sheep. This may be because lying has a surprisingly high mean MMI in this dataset, despite being an inactive behaviour.

The k-means clustering method resulted in a categorisation, but these categories were not clearly defined by specific behaviours. They included observations of various individuals and multiple behaviours, while some contained records from only one animal. This is likely because k-means clustering is extremely sensitive to outliers (Olukanmi & Twala, 2017), which indicates that the individual ewe and lambs that were in clusters alone probably had very different AX3 outputs from other in the flock. Because welfare data was not being collected at the time of validation observations, it is not possible to say whether these animals were outliers because of an underlying condition. However, one month after the validation observations, lamb 13859, a k-means outlier, had a strongyle FEC of 1881 epg. Given that eggs do not become apparent in faeces until at least three weeks after initial infection, it is possible that this lamb was singled out as an outlier by k-means because of its behavioural expression being affected by early strongyle infection. In future applications these outliers may be the datapoints to trigger alerts in PLF tools since they may signal different behavioural expressions caused by welfare issues. Further research on a higher number of infected lambs would be necessary to confirm this hypothesis.

While the clusters reported did not result in a complete validation of AX3 for identifying separate behaviours, they did indicate that this method has the potential to do so with an improved dataset. The mean value of y describes the height of an AX3 on the sheep's neck during behaviours, so the 7 clusters based on this value are somewhat logical. Lambs grazing have their collar lower to the ground than ewes grazing, so cluster 1 has a lower mean y than cluster 3. Lambs standing in turn have higher heads than lambs grazing, so cluster 2 has a higher mean y than cluster 1, and so on. By basing the clusters on mean y, only one dimension of tri-axial accelerometers was being exploited, and behaviour was not entirely being described. Movement forward and back, which would be described by the x and z axes, was not included in the analysis. This made identifying behaviours like locomotion impossible. This may be possible with a more variable dataset. Future studies would need to ensure that validation datasets capture a wide range of behaviours, which could be achieved by observing more sheep, or observing the same amount of sheep over longer periods of time at different times of day. For example, more lying behaviour could be captured through nightly observations. It is worth noting that this study combined eating and drinking behaviour in its ethogram. This was done because drinking from troughs is extremely rarely recorded and indeed, it was never recorded in this study. However, if it had been recorded, it could not have been treated as the same behaviour as grazing for the AX3 validation, as the profiles of both behaviours from the accelerometers would likely be different.

Location of the sensors on an animal's body has been reported to impact the quality of results obtained (Barwick et al., 2018). In a study using accelerometers to detect lameness, sensors taped onto sheep's legs detected lame walking with 87% accuracy, while sensors on ear tags had 82% accuracy and on a collar had 35% accuracy (Barwick et al., 2018). Other studies have reported up to 95% accuracy in activity classification by accelerometers in collars, with a high level of agreement (Cohen's weight K>0.80) between sensors on ears and in collars (Walton et al., 2018). These publications highlight that placement may be an important factor in validating accelerometers for different purposes. Sensor placement must therefore be carefully considered when developing PLF tools. It is possible that the use of collars around sheep's necks in the present study could explain the high MMI of lying. Despite the body remaining still during lying, the head and neck could be quite active and the AX3 data may have reflected this.

HMM was suggested by Dr Juan Morales, an ecological statistician with experience in applying machine learning to animal behaviour datasets. After reviewing my attempt at building an HMM, Dr Morales indicated that my validation dataset was not suited to this approach due to its low level of variation. He suggested that the machine learning technique of deep neural networks may be a rigorous tool to use. This was noted and appreciated, but due to a lack of skills in this technique and a lack of time to acquire them, it was decided that this approach was beyond the scope of this project. Past studies indicate that random forest algorithms are particularly useful for behavioural data, so these should be prioritised in future research (Barwick et al., 2018; Busin et al., 2019; Mansbridge et al., 2018).

4.5 Conclusion

The various approaches applied to this AX3 validation dataset illustrate the complexity of validating new technologies in real, commercial, extensive farming conditions. This should not render this step of technology research and roll-out any less crucial. Differences in behaviour when wearing sensors should always be tested to avoid misinterpretation of study results and negative animal welfare impacts. The fact that k-means clustering resulted in an inconclusive but understandable categorisation of behaviours based on mean y value of AX3 suggest that unlabelled machine learning has potential to validate PLF tools.

Chapter 5: Using Bluetooth beacons to examine ewe-lamb distance as an indicator of compromised ewe and lamb welfare

5.1 Introduction

Precision Livestock Farming (PLF) can monitor health, production and welfare indicators in livestock through wearable sensors or non-invasive technology in barns such as cameras (Berckmans, 2017; Larsen et al., 2021). Wearable sensors have the advantage of collecting individual information rather than group-level measures, which is a guiding principle of PLF (Berckmans, 2017). They are also more practical in extensive environments where animals can cover large distances and infrastructure-mounted technology like cameras are unrealistic (Rutter, 2014). They can then provide timely diagnosis of diseases for otherwise mostly unsupervised animals, in turn reducing financial losses and improving welfare (Neethirajan, 2017). To be functional on outdoor pastures, PLF tools must be robust, they must have an adequate power supply and long-range data transmission is preferable (García García et al., 2023; Terrasson et al., 2016). Bluetooth Low Energy (BLE) technology is a potential tool to collect location information from animals without the high prices and low battery life that burden Global Positioning System (GPS) tools. Bluetooth has successfully been used to monitor activity and falls in humans (Chan et al., 2013). In sheep, it has been used to monitor ewe-lamb distance to identify maternal pedigree (Sohi et al., 2017) and to study social contact frequency in sick lambs (Morris et al., 2022). I believe it has potential as a welfare monitoring tool since it can reliably measure interactions and distance between two individuals (Morris et al., 2022; Sohi et al., 2017; Walker et al., 2023). This means it could monitor ewe-lamb distance as a proxy for the maternal relationship dynamics (O’Connor et al., 1985; Pickup & Dwyer, 2011). In the UK, hill breeds often lamb outdoors and without human intervention, so a strong ewe-lamb bond is crucial to lamb survival (Dwyer, 2008). Bluetooth technology could allow for measurements of ewe-lamb distance in real time that could serve as a proxy for monitoring the ewe-lamb bond. Mutual recognition and awareness between ewes and lambs maintain the ewe-lamb distance (Pickup & Dwyer, 2011), so BLE beacon systems could alert farmers to changes in ewe-lamb distances that may indicate a welfare concern affecting these aspects of the maternal relationship. For this purpose, the effects of various welfare concerns on ewe-lamb distance must be examined.

Lamb survival depends on the expression of bonding behaviours by the ewe and lamb and these interactions evolve as the lamb matures (Dwyer, 2014; Nowak et al., 2000). The ontogeny of ewe-lamb distance progresses from the lamb remaining very close to the ewe in the first week of life, followed by an increase in ewe-lamb distance until 6 weeks post-partum, at which point it remains steady while the lamb starts joining peer groups up to 100 m away between seven and nine weeks of age (Arnold & Grassia, 1985; Pickup & Dwyer, 2011). Indeed, sheep go through a period of bonding immediately following birth, characterised by the ewe licking and grooming the lamb, accompanied by low-pitched bleating (Alexander, 1988; Hersher et al., 1963). Following this bonding period at parturition, ewe-lamb interactions consist of frequent sucking interactions, a close spatial relationship, and maternal vigilance (Dwyer & Lawrence, 2005). Once the olfactory bond is established between dam and offspring, short periods of separation (24 hours or less) will not have any negative effects on maternal behaviour or lamb acceptance by the ewe (Lévy et al., 1991). Close ewe-lamb distance can improve maternal protection from predators (Hewson & Verkaik, 1981), facilitate sucking and could help establish food preferences in lambs and encourage observational learning (Black-Rubio et al., 2007; Saint-Dizier et al., 2007).

Individual and breed differences exist in the expression of maternal behaviour in sheep (Dwyer & Lawrence, 2005; Dwyer & Lawrence, 2000; Dwyer & Lawrence, 1999). Individual differences can arise based on breed, parity, ewe temperament, stress and nutrition during pregnancy and lamb behaviour (Dwyer, 2008a, 2014). A study using embryo-transfer to manipulate the ewe-lamb relationship in Scottish Blackface sheep, a hill breed, and Suffolk sheep, a lowland breed, found that Blackface ewes stayed closer to their lambs, regardless of the lamb's breed, with a mean distance of 6.10 metres, than Suffolk ewes whose mean ewe-lamb distance was 11.54 metres (Dwyer & Lawrence, 1999). Despite the knowledge that all the above factors affect the spatial relationship between ewe and lamb, it is unclear if or how the welfare states of ewe or lamb may have an impact. This study aims to explore the relationship between welfare measures and ewe-lamb distance recorded by BLE technology. I hypothesise that 1) lambs affected by welfare issues will have larger ewe-lamb distances than those without welfare issues as lambs may express sickness behaviour or other impairments that may make them less able to keep up with the ewe's movements and that 2) ewes suffering from welfares issues will also increase their ewe-lamb distance as they will be less likely to respond to the lamb compared to ewes without welfare issues.

5.2 Methods

5.2.1 Ethical approval

Ethical Approval was obtained from the SRUC's Animal Experiment Committee for trial number SHE AE 10-2022.

5.2.2 Animals

Thirteen Scottish Blackface and 23 Lleyn ewes and their 73 lambs (3 singles, 29 sets of twins, 4 sets of triplets) kept on pasture at Kirkton Hill Farm in Scotland were the subjects of this study. There were 30 female lambs and 33 males, tails were not docked and males were not castrated. The ewes were placed in a lambing field starting from 2 weeks prior to expected lambing dates, and they remained in the lambing field for the 6-week duration of this study. Lambing began on April 15th, 2022. Management checks occurred twice daily (morning and afternoon) at the height of lambing (first four weeks) then were reduced to once daily. Lambs were tagged and weighed within 24 hours of birth during management checks. No castration or tail-docking occurred. Data collection occurred in three phases. Phase 1 ran from April 22nd to May 2nd. Phase 2 ran from May 6th to 20th, and Phase 3 ran from May 24th to June 6th, 2022. Phase length was defined by the battery life of technological tools. The lambing field was fenced, measured 3 hectares and contained one drinker. It was a sloped field of permanent pasture at an elevation of approximately 190m. Hay was provided for the ewes in Phase 1 and sheep were moved off the lambing field between Phases 2 and 3 to allow the grass to recover.

5.2.3 Experimental Design

In Phase 1, 23 ewes wore collars (Figure 5.1) containing a Feasybeacon BLE beacon (Feasycom, Shenzhen, China) and a Wearable Integrated Sensor Platform (WISP) reader, specified and commissioned by SRUC and designed, built and programmed by CENSIS (Glasgow, UK). These readers acted as receivers for the BLE beacons by communicating with a Wirnet iStation low-power wide-area network (LPWAN) gateway (Kerlink, Thorigné-Fouillard, France), installed 625 metres away. Two versions of the same beacons were used: the BLE 5.1 (3000 series) and Nordic beacons (4000 series). The ewe collars weighed 333 grams on average and were made of webbing sewn between buckle fasteners onto which the WISP readers were fixed using cable ties. In Phase 2, 17 ewes had collars and in Phase 3, 14 ewes wore collars. The reasons for the decreasing numbers of collars being worn were that some WISP readers had to be sent to another research project in Phase 2, and eight ewes developed lesions due to the collars, so they were removed permanently. Indeed, animal numbers in this study were limited by the number of collars available for ewes to wear, since these were custom-made pieces of equipment. Only ewes expected to have twins based on scanning results were fitted with collars. In reality, 50 twin lambs, three singles and one triplet whose siblings were moved to the pet lamb pen were fitted with a BLE beacon in a collar around their necks (Figure 5.1) within 24 hours of birth, at the same time as tagging and other management actions described above. Of these lambs fitted with collars, 30 were males and 24 were females. The lamb collars weighed 25 grams on average and were made of stretchy webbing, Velcro® patches and a plastic tag on which to write an ID number. All the materials to make ewe and lamb collars were purchased from online retailer Amazon (Seattle, USA). Animals were individually marked and numbered with livestock marker paint (Ritchey Livestock ID, Brighton, USA) for easy visual identification.

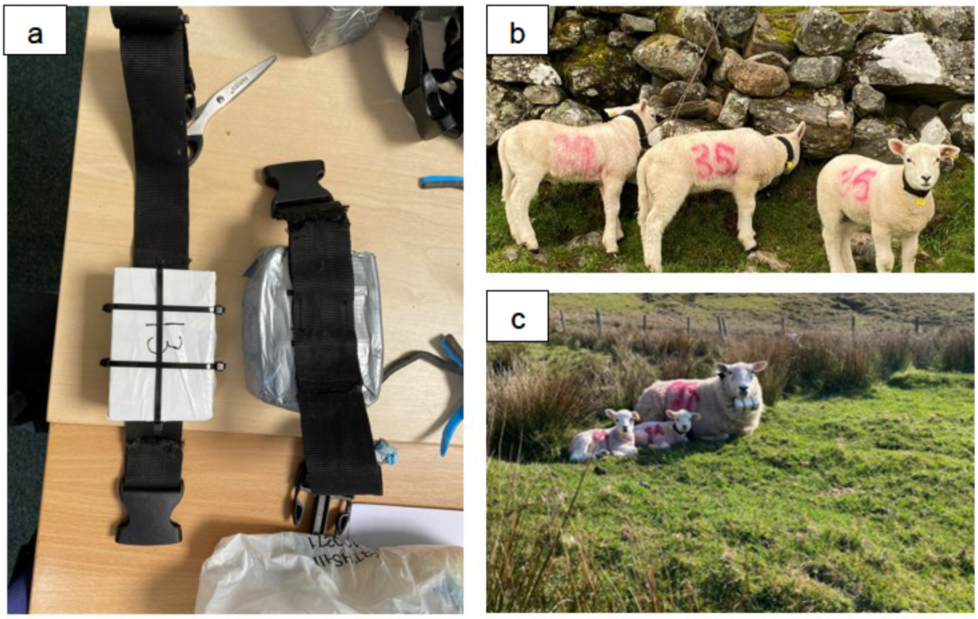

Figure 5.1. Ewe collars (a), lamb collars (b) and collars on ewes and lambs in the field (c).

Natural infection with lameness, parasites and other welfare conditions was allowed to occur, knowing that some animals would be affected more severely than others. The authors relied on this natural variation of infection to create variation across the sample group. Ewes and lambs were checked daily by technicians for signs of lameness, scouring, and mastitis (using standardised scores described in Table 3.1), as well as to identify and manage any lesions caused by the collars. Sheep were gathered and weighed monthly by passing through races leading to a weigh-crate incorporated into a Prattley 5-way Auto Draft (Prattley Industries, Temuka, New Zealand) with Tru-test™ MP600 load bars and fitted with a Tru-test™ XR3000 weigh head (Tru-test Group, Auckland, New Zealand). Close inspections of necks were carried out during weighing events, with Terramycin™ cutaneous spray (Zoetis UK Limited, Leatherhead, UK) administered where any lesions were visible. If ewes had lesions caused by the collars, they were permanently removed. All collars were removed during these weighing events, technology was charged and re-attached three to four days later by repeating the gathering procedure. An endpoint for oral anthelmintic treatment with Oramec drench at a dose of 2.5ml per 10kg of body weight (Boehringer Ingelheim International GmbH, Ingelheim, Germany) was set when lambs or ewes had a dag score of 3 or more on the scoring scale described in Table 3.1 or if ewes lost 15% of their weight within two weeks. Treatment did not exclude sheep from the study. Lameness was treated with Terramycin™ cutaneous spray (Zoetis UK Limited, Leatherhead, UK) when sheep were gathered.

5.2.4 Data Collection

5.2.4.1 In-field Welfare Assessment

Every Friday morning, an in-field welfare assessment was conducted on all ewes and lambs using the same scoring system as was used in Chapter 3, Table 3.1. Each animal was observed from a distance and assigned an in-field dag score, fleece score, and lameness score.

5.2.4.2 PLF Technology

The WISP readers on ewes' collars sent information to a database over a LPWAN network every 5 minutes. This dataset consisted of the identity of the 16 nearest beacons and an averaged Received Signal Strength Indicator (RSSI) value for each of these 16 neighbours over the last 5 minutes. RSSI is a continuous negative numerical value indicating relative distance between the beacon and the reader. The lower the RSSI, the further away the beacon was from the reader. The closer to 0 the RSSI, the closer the beacon was to the reader. Validation of the BLE devices to convey actual distance had previously been carried out (Walker et al., 2023).

5.2.5 Data Analysis

All data processing and analysis was conducted in R version 4.2.3 (R Core Team, 2023) via R Studio (version 3.0). Data from the three phases were combined and analysed together. The package *tidyverse* (Wickham et al., 2019) was used to conduct raw data processing. The raw dataset was filtered to only include days where welfare assessments had taken place (6 days in total) and only readings between ewes and their own lambs, which were the only observations relevant to studying ewe-lamb distance. All lameness and dag scores other than 0 were combined to a single score of 1, to signify there was a welfare concern recorded. The processed dataset was analysed using the package *glmmTMB* for Gaussian Generalised Linear Mixed Models (GLMM) to examine the effect of lameness, dag score and fleece score on ewe-lamb distance. RSSI was converted to distance in metres using the equation created for this purpose by Walker *et al.* (2023):

*Predicted distance = 2.71828^(-4.678276 – (0.096160*RSSI))* for the BLE 5.1 (3000 series) and

*Predicted distance = 2.71828^(-5.082263 - (0.097676*RSSI))* for the Nordic (4000 series) beacons.

Ewe-lamb distance transformed by natural log was the response variable and ewe dag score (0,1), lameness score (0,1), fleece score (0,1), ewe breed (BF=Blackface, LY=Lleyn) and observation week (1-6) were explanatory variables. Ewe ID was included as a random effect. There was too little variation in the lamb welfare data to include them in the models. Model fit tests and pairwise comparisons were performed as described in 3.2.5.2.

5.3 Results

The filtering of observations relevant to ewe-lamb distance from the raw BLE dataset resulted in only four families (a family consists of one ewe and her twin lambs) being included in week 1, nine families in week 2, 13 in week 3, 12 in week 4, three in week

5 and 11 in week 6. The low but increasing numbers of families included between weeks 1 and 4 occurred because lambs were being born during the observation period and their families entered the dataset as they were born. The very low number of families in week 5 resulted from a malfunction in the technology, causing only three WISP readers to communicate data that week. Most WISP readers came back online for the final week, but two remained broken. Eight ewes had minor injuries from the collars at the end of Phase 2, so they were removed from their necks permanently. Seven lamb collars fell off throughout the study and only two were ever recovered. The data from these collars were used until the last recorded date of observation of the collar on a lamb. Figure 5.2 shows the number of transmissions of BLE data over the 6 days of welfare observations, which were received over 24 hours each day. Lower counts or blanks indicate a failure of the WISP readers to communicate their data in real time. The variation in count is due to the varying number of ewe collars successfully communicating with their lambs' beacons and sending data.

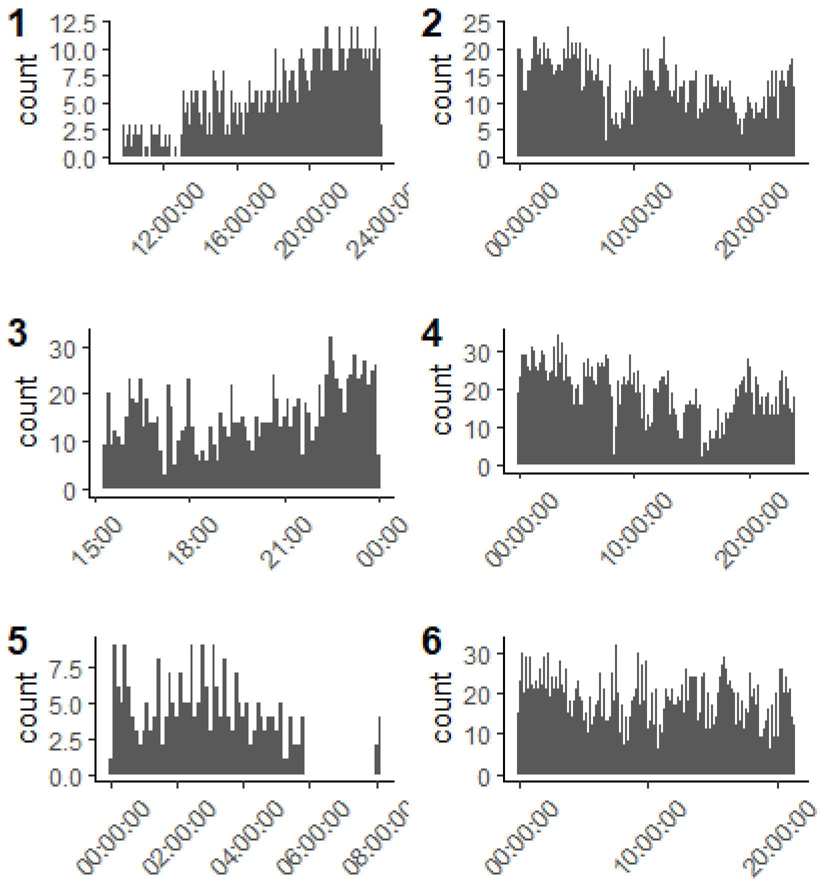

Figure 5.2. Connection counts between WISP readers and beacons communicated to the LPWAN gateway over the six days of welfare observation.

All lamb welfare scores remained at 0 throughout the entire observation period, meaning no lamb was ever observed scouring, lame or with any fleece problems. Overall, 15 of 23 ewes wearing collars were affected by 32 counts of welfare concerns over the 6 weeks of observation (Table 5.1). The number of ewes affected by two welfare concerns simultaneously varied over time from one in week 3, to four in week 4, seven in week 5 and two in week 6. No ewes were recorded as being affected by the three conditions measured simultaneously.

Table 5.1. Number of ewes with a dag, lameness or fleece score of 1, indicating a welfare concern, by week of observation.

	Week 1	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Total ewes affected over entire study period***
# of scouring ewes	0	0	3	5	2	0	7
# of lame ewes	0	3	4	4	2	2	10
# of ewes with fleece issues	0	0	0	3	1	3	5
Total # of welfare issues	0	3	7	12	5	5	

*These totals only count each ewe once for each welfare concern, meaning it is not the sum of counts from weeks 1 to 6.

Ewe-lamb distance as measured by the technology ranged from 0 to 50 m, with a median of 5m (IQR=11m). There was little variation in the spatial relationship between ewes and lambs over time (Figure 5.3), although mean ewe-lamb distance was lower in week 1 (7.22±2.95m) compared to week 2 (8.23±3.14), week 3 (8.35±3.07), week 4 (8.44±3.15), week 5 (8.38±3.05) and week 6 (8.28±3.11)($p<0.001$) (Figure 5.3).

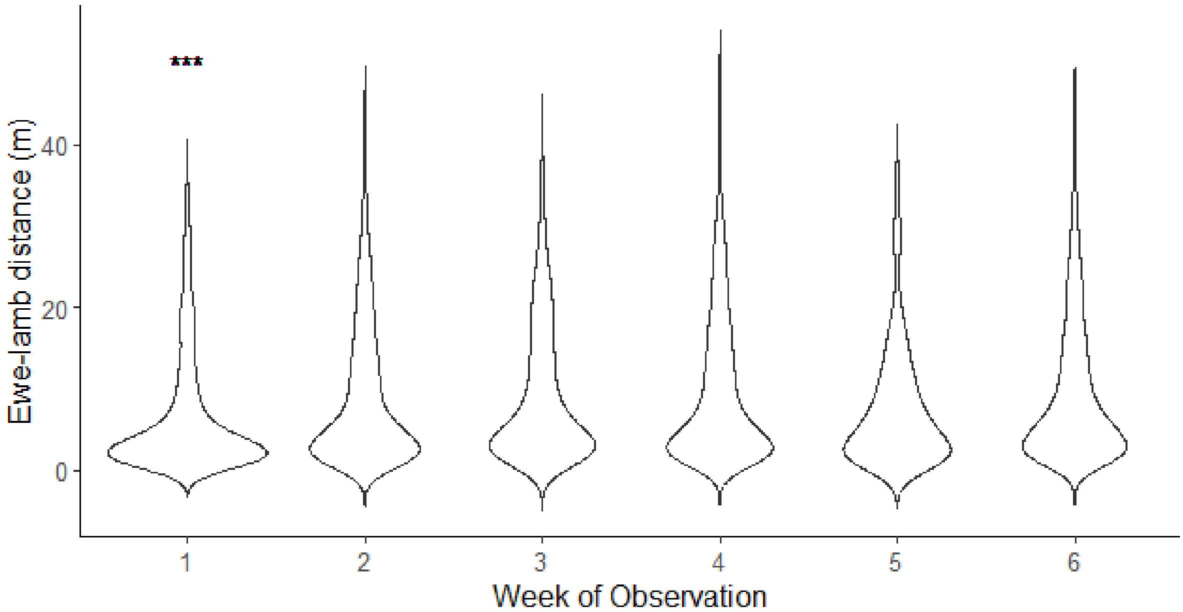

Figure 5.3. Violin plot of ewe-lamb distance in metres as measured by the BLE beacons over the six weekly days of observation, where the mean ewe-lamb distance from the first day of observation is significantly different from the other five days.

Dag score ($X_{2(1,12289)}$=0.42, p=0.519) and ewe breed ($X_{2(1,12289)}$=0.06, p=0.799) had no effect on ewe-lamb distance. The range of ewe-lamb distances reported was the same for both breeds. Ewes with no fleece problems (score 0) had a higher mean ewe-lamb distance (8.73m±1.05) than ewes with fleece problems (score 1) (7.03m±1.07)($X_{2(1,12291)}$=33.89, est=0.217±0.04, t=5.82, p<0.001) (Figure 5.4). Sound ewes had a higher mean ewe-lamb distance (8.73m±1.05) than lame ewes (7.03m±1.04)($X_{2(1,12291)}$=57.66, est=0.094±0.01, t=7.61, p<0.001)(Figure 5.4).

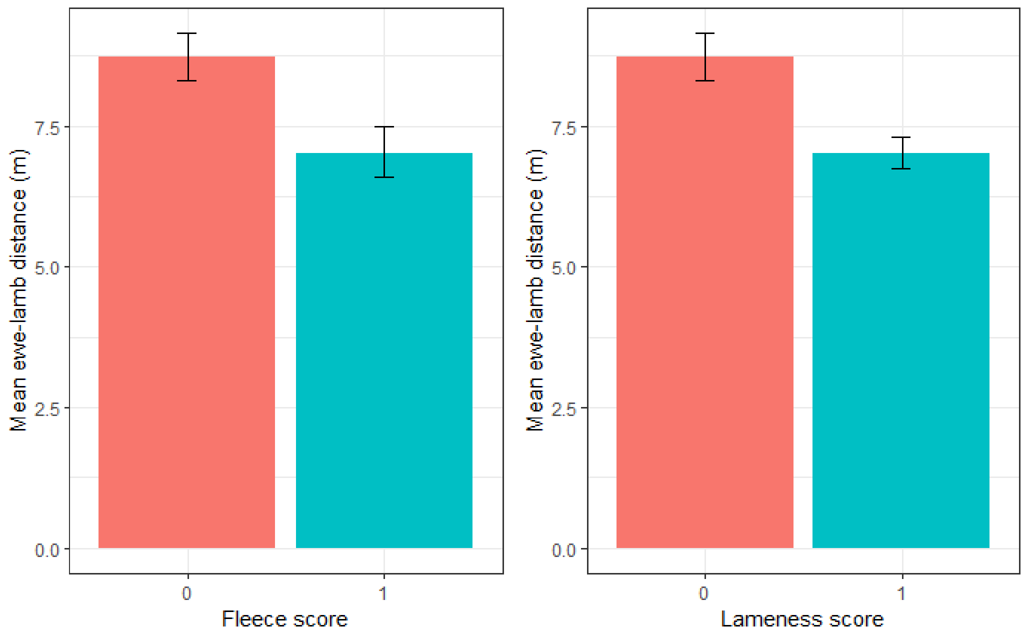

Figure 5.4. Mean ewe-lamb distance of ewes with fleece scores 0 (healthy fleece, no issues) and 1 (any fleece problem visible) and of sound ewes (score 0) compared to lame ewes (score 1).

5.4 Discussion

These findings lead us to reject the second hypothesis that ewes experiencing welfare issues would have increased ewe-lamb distances. The hypothesis was based on the assumption that sick ewes would display sickness behaviour patterns, therefore reducing their investment in social and possibly maternal behaviour and increasing apathy (Dwyer & Bornett, 2004; Hart, 1988). The opposite relationship was found. Lame ewes and ewes with fleece problems were closer to their lambs than healthy ewes. This differs from Chapter 3 results, where welfare problems were not associated with ewe-lamb distance. However, distance was visually estimated in Chapter 3, whereas the BLE beacons in this study may have provided more accurate measurements. The association between ewe welfare and ewe-lamb distance could be due to the behavioural cues that govern the ewe-lamb relationship. Lambs rely on visual, vocal and behavioural traits to locate and approach their dam (Nowak et al., 2000). Previous research has shown that ewes likely control the dynamics of the spatial relationship despite lambs having an increasing effect on it as they age (Arnold & Grassia, 1985; Dwyer & Lawrence, 2000a; Pickup & Dwyer, 2011). Ewes may impact lambs' continued interest in remaining near them in later lactation through their reactions to sucking attempts, thus influencing ewe-lamb distance (Arnold et al., 1979; Pickup & Dwyer, 2011). Lambs seem to respond specifically to their dam's calls for them to approach from one month of age onwards (Shillito & Hoyland, 1971) and most sucking interactions are preceded by ewe vocalisations (Dwyer & Lawrence, 2000a). Ewes also encourage lambs to approach for sucking through a vigilance, or head-up posture (Dwyer & Lawrence, 2000a). This previous research shows that ewe behaviour, posture and vocalisations control lamb movements and therefore ewe-lamb distance (Dwyer & Lawrence, 2000a; Pickup & Dwyer, 2011; Shillito & Hoyland, 1971). It may be that certain welfare conditions, such as lameness, directly or indirectly modulate these cues, leading lambs to approach their dam more often. For example, lame sheep often display head nodding or flicking during locomotion (AWIN, 2015; Barwick et al., 2018). It is possible that head nods from lame ewes could be interpreted by lambs as the head-up posture encouraging them to approach.

Ewe-lamb proximity fluctuates throughout the day, although this should not have affected our results since beacon measurements were sent throughout the day (Sohi et al., 2017). The stable and relatively low mean ewe-lamb distance over the six weeks was in line with past studies which found that lambs remain close to their dams until between 7 and 9 weeks of age, when the ewe-lamb distance increases as lambs spend more time in peer groups (Arnold & Grassia, 1985). The finding that lambs were significantly closer during the first week post-partum aligns with the fact that ewe-lamb distance is the lowest during early lamb life (Arnold & Grassia, 1985; Pickup & Dwyer, 2011; Pickup, 2004). However, the lack of a significant increase in ewe-lamb distance following this first week of life differs from past findings (Pickup & Dwyer, 2011). Previous studies have found that through increasing their interruptions of sucking behaviour and reducing their active approaches towards their lambs, ewes contribute to the steady increase in ewe-lamb distance after 4 weeks post-partum (Pickup & Dwyer, 2011). Despite known breed effects on ewe-lamb distance between lowland and hill breeds, no differences were seen across ewe breeds in this study. Both breeds used, Lleyn and Blackface sheep, are hill breeds. This likely explains the lack of a breed effect, since breed differences in ewe-lamb distance were only reported between lowland and hill breeds (Dwyer & Lawrence, 1999).

It is possible that ewe-lamb distance was influenced by the underlying cause of the fleece loss in ewes. Fleece score records the degree of fleece cover on the body, taking into account any areas of loss, thinning, or shedding (AWIN, 2015). Ectoparasites (e.g. mites, flies) lead to scratching, biting and rubbing that can result in patchy fleece loss (AWIN, 2015; Plant, 2006). Stress and nutritional imbalance can cause weakness in the wool structure, leading to breaks and shedding (AWIN, 2015; Dixit et al., 2011). Additionally, rough handling may cause wool pulls (AWIN, 2015). A previous Bluetooth beacon study observing ewe-lamb pairs for 40 days after lambing reported that ewes with a high percentage of lying or inactive behaviour had more contact with their lambs (Lewis et al., 2023). Psychological stress due to negative human interactions during pregnancy has been associated with more time grooming lambs after birth (Hild et al., 2011) and a greater motivation to remain with lambs in the presence of humans (Roussel et al., 2006). This could be one explanation for the present study's results: that psychological stress has caused fleece loss and closer proximity between ewes and lambs. Since many causes of ewe fleece loss, such as undernutrition and stress, are also associated with poor maternal behaviour (Dwyer, 2014; Dwyer et al., 2003; Kiley-Worthington, 1977; Putu et al., 1988), it is difficult to support a direct causation between fleece loss and smaller ewe-lamb distances, which are usually associated with high maternal behaviour scores (O'connor et al., 1985). Future analyses comparing ewe-lamb distance before and after the ewe's welfare was challenged might clarify any causal links behind the associations reported in the present study.

It was impossible to test the first hypothesis, that lambs with welfare concerns would have higher ewe-lamb distances, because no lambs in the study were affected by the welfare conditions being studied. Relying on natural infection allows experiments to illustrate commercial conditions and avoid unnecessary welfare compromise in study animals with induced infections. However, there is always a risk that the desired challenge will not occur to a level significant enough to study its effects. It results in more variable infection results and often requires large amounts of animals to reach statistical significance (Colby et al., 2017). Further studies on pastures known to be infected with high levels of *Dichelobacter nodosus*, the causal agent for scald and footrot, could address this shortcoming. Longer studies that observe lambs into the eighth week of life and beyond could have provided more opportunities to observe gastrointestinal parasite infections, as this is the age lambs start grazing more and ingesting more larvae (Donald & Waller, 1973).

Some challenges arose from the use of new technology. The LPWAN connection was sometimes lost, and data stopped being collected for small periods of time. Some collars were lost or damaged. Though these challenges are to be expected in a proof-of-concept trial, they would need to be resolved before the technology could be commercialised for farmers. The large datasets that result from collecting data in real time lend themselves well to machine learning algorithms, which can be trained and tested on the data resulting from a small trial such as this one. The algorithms could quickly identify patterns of behaviour to act as early indicators of poor welfare and alert users to the issue. However, these types of technologies must be validated and rooted in biological reality. Additionally, some collars caused lesions on ewes' necks and needed to be removed. Results from Chapter 4 indicated that even when no lesions are caused, collars may have an impact on sheep behaviour and welfare. It is therefore possible that ewe behaviour was modulated by wearing the collars, potentially affecting this study's results.

5.5 Conclusion

This study found an association between ewe-lamb distance and ewe welfare scores related to fleece quality and lameness, unlike the study in Chapter 3, where no such relationship was present. Further studies examining indicators of lamb welfare are needed to complete the picture of ewe-lamb behavioural relationship. Ewe-lamb distance has potential as an animal-based welfare indicator that could be measured by PLF in extensive farming systems although more research is required.

Chapter 6. Norwegian sheep farmers' perception and use of Precision Livestock Farming (PLF) technologies

6.1 Introduction

Through digital or Precision Livestock Farming (PLF) technology, farms are capturing more data than ever in the hopes of improving decision-making and efficiency (Klerkx et al., 2019; Rotz et al., 2019). New commercial products are developed and marketed to farmers every year (Sundmaeker et al., 2016). Some offer increased efficiency, such as the Internet of Things, while others replace human labour in physically demanding tasks, like milking robots on dairy farms (Hansen, 2015; Wolfert et al., 2017). Others, for example Global Positioning System (GPS) collars for improved surveillance of animals in outdoor systems, offer access to previously unattainable information such as monitoring animal behaviour and location, thus supporting farmers in their decision-making (Neethirajan & Kemp, 2021a; Wolfert et al., 2017). Despite the undeniable advantages technology can bring, it is unclear how often the development of new tools is centred around farmers' needs and means, rather than the commercial drivers for the technology companies (Lesser, 2014; Wolfert et al., 2017). The users still face challenges and varied experiences when applying PLF on their farms. These can range from practical issues such as rodents damaging new wires installed in barns, to more intangible challenges such as the increased mental burdens involved in the managing and interpretation of large datasets (Banhazi et al., 2015; Hostiou et al., 2017). Additionally, sheep products from extensive outdoor systems are often perceived by consumers as being more "natural" in comparison to products from intensive, indoor systems (Goddard et al., 2006), and it is unclear how accepting consumers will be of high levels of technology being used in these "natural" environments (Wathes et al., 2008). Although it is unrealistic and unfair to expect important time commitments from farmers to participate in the development of technology, they should be consulted, and their needs must be understood.

Much research has been conducted on the factors affecting acceptance of technology by farmers. The Technology Acceptance Model (TAM) identified perceived usefulness (PU) and perceived ease of use (PEOU) as accounting for up to 70% of variation in farmer behaviour around use and acceptance of technology (Davis et al., 1989; Flett et al., 2004). A paper applying Normalisation Process Theory (NPT) (May et al., 2007; May & Finch, 2009; May et al., 2009) to understand British sheep farmers' adoption of PLF technology reported that it helped the authors understand the context preventing adoption (Kaler & Ruston, 2019). NPT is a conceptual tool for understanding and explaining the social processes through which new practices of thinking, enacting, and organising work are put into place (May et al., 2009). It is helpful when seeking to understand successes and failures in implementation, which was useful when studying PLF adoption by sheep farmers (Kaler & Ruston, 2019; May et al., 2007). The authors concluded that farmers believed that PLF was costly, difficult to implement, and posed a threat to their role as good stockpeople (Kaler & Ruston, 2019). Lima *et al.* (2018) studied the use of EID as a PLF management tool rather than simply a mandatory animal identification system to explore beliefs and practices affecting British sheep farmers' PLF adoption rates. Non-adopters were more likely to believe that governments pressure farmers into adopting technology while adopters had higher information technology literacy and intended to intensify production in the future (Lima et al., 2018). A Norwegian study found that a strong belief in technology, high wage rates and difficulties getting skilled labour led to high adoption of milking robots in certain regions (Hansen, 2015). However, beyond adoption, there are few studies asking about the experiences and satisfaction levels of current users of PLF technology in animal agriculture.

The regular use of technology in farming gives rise to ethical debates, notably about the impact it can have on animal welfare and the human-animal relationship (Cornou, 2009; Wathes et al., 2008). Researchers and farmers alike have identified the risk of reducing farmers' contact with their animals (Hartung et al., 2017; Hostiou et al., 2017; Schilling et al., 2008). A review paper identified 12 potential threats to animal welfare created by PLF technology, grouped into four categories: i) direct harm, e.g. through a PLF malfunction due to a power cut, ii) indirect harm via the user, e.g. over-reliance on PLF to detect welfare concerns could reduce users' abilities to do so themselves, iii) indirect harm via transformations in animal farming systems, e.g. building a barn according to PLF needs rather than animal needs, and iv) indirect harm via changes to the moral status of animals in society, e.g. PLF causes more animal objectification and reduces society's ability to see each animal as an individual (Tuyttens et al., 2022).

Thus, there is a need to understand the lived experiences of farmers using technology to be able to assess the risks and recognise the benefits of PLF.

The Norwegian sheep industry is an interesting case study to investigate farmers' beliefs about technology. Firstly, many livestock technology tools were invented in Norway, for example Nofence© (Batnfjordsøra, Norway), a virtual fencing system that allows users to draw digital borders that their livestock cannot cross when wearing their collar, or Findmy (Kvikne, Norway) and Telespor AS (Asker, Norway), two kinds of GPS collars that record the location of sheep at a frequency chosen by the user. Secondly, Norway has a very widely digitised food production system. For example, Norwegian dairy farmers have the highest rate of adoption of milking robots in the world (Hansen, 2015). Despite anecdotal evidence that Norwegian sheep farmers adopt technology at a high rate, the sheep sector is seldom at the foreground of PLF technology discussions in Norway.

This study used semi-structured interviews to explore the opinions of Norwegian sheep farmers who are current users of PLF. Using thematic analysis (Braun & Clarke, 2006, 2019), I aimed to understand farmer motivations for using PLF beyond PU and PEOU, and the barriers they may face in its implementation. The goal was to inform future PLF development and applications in the small ruminant sector from a farmer perspective.

6.2 Methods

Ethical approval was obtained by the University of Edinburgh's Human Ethical Research Committee (HERC_2023_087) and no ethical approval was required from Norwegian institutions.

6.2.1 Interviews with sheep farmers

Farmers were recruited through local sheep farmer organisations *Agritech Cluster-Agriforsk* and *Arktisk kompetansesenter for Sau*, and through the Norwegian Institute for Bioeconomy Research (NIBIO) in September 2023. The selection criteria for participants were that they had more than 20 ewes and used any kind of digital technology in their day to day running of the farm (e.g. Radio Frequency Identification (RFID) wands, automatic feeding systems, GPS collars). Interviews occurred until saturation was reached. The saturation criteria used was *Code Frequency Count* (Hennink et al., 2017, 2019), meaning that no or few new codes in successive transcripts were identified (Hennink & Kaiser, 2022; Krueger & Casey, 2015). Participants gave written consent after reading a participant information sheet that contained a summary of study objectives, the interview process, data management, anonymity, and confidentiality. These documents were offered in English and Norwegian. Interviews were conducted in English in person at the farmers' homes, workplaces or at public sheep farming events by one researcher. The interviews lasted between 30 and 90 minutes. Interviews were recorded using a high-resolution WAVE/MP3 recorder (R-09HP by Roland Corporation, Shizuoka, Japan) and an iPhone (iPhone 12 Mini by Apple, Cupertino, USA) as a back-up. A semi-structured interview guide (Supplementary Materials 6.6.1) was used to direct conversations during the interviews. The interview guide consisted of four sections: i) general description of the technology used on farm, ii) the advantages and disadvantages of the technology, iii) their perceptions of technology across the farming industry, and iv) their vision for the future of technology in sheep farming. At the end of the interviews, farmers were asked if there was anything not discussed that they wished to add.

6.2.2 Participants

In total, 19 interviews were conducted with 23 farmers (during four interviews, I spoke to a couple or a multigenerational team running the farm together). Three counties of Norway were covered: Nordland, Trøndelag and Møre og Romsdal. Participant age ranged from 35 to 70 with a median age of 53. There were seven women and 16 men interviewed. Flock size ranged from 20 to 400 ewes, with a median flock size of 140. Across all the participants, six sheep breeds were farmed: Norwegian White Sheep, Gammalnorsk, Grå Trøndersau, Steigarsau, Norwegian Pelssau and Spælsau.

6.2.3. Analysis of interview recordings

Interview recordings were transcribed first using the Otter.ai transcription software (Otter.ai, Mountain View, USA). The transcripts were then validated by the authors. An inductive, reflexive thematic analysis using the methodology described by Braun and Clarke (2019) was carried out. The inductive approach relies on the data themselves to identify themes, rather than analysing data with a pre-conceived framework in mind. Semantic coding was the utilised approach, which means the analysis of themes was based solely on participants' words shared during the interview. Latent coding was also carried out. Latent coding refers to the interpretation of the meaning behind the words used to identify underlying assumptions or ideas (Braun & Clarke, 2006). The analysis was conducted through a constructivist lens that places emphasis on the individual's role in constructing knowledge while acknowledging social influences, past experiences and existing knowledge (Fosnot, 2013; Moallem, 2001). Given that the interviews were conducted by a woman younger than all the participants, who were mostly men, the authors acknowledge the gender and age dynamics at play. As a researcher, the interviewer may have influenced what participants expressed during the interviews. This may have additionally been affected by the language barrier, as farmers were speaking in their second or third language. A coding guide was created by reading through the transcripts where salient data extracts were selected using NVivo 14 software (QSR International, Burlington, USA). Coded text was organised into themes, which were reviewed and refined. The relationships between themes were mapped and final themes were identified.

6.3 Results

6.3.1 Technology

Participants spoke about 10 farming technologies across all the interviews. The most popular were collars providing sheep location via GPS through the mobile telephone network or satellites. In order of prevalence among our participants, other technologies included registration software, virtual fencing collars, RFID wands, cameras over lambing pens, Bluetooth connected weigh crates, drones, automatic feeders, ultrasound scanners, and automatic feed mixers.

6.3.2 Themes

Five themes were extracted from the transcripts. The first was entitled *Resources and Savings*. This theme covered the economic and time costs and savings that motivated farmers to use technology (e.g. increased profits) or acted as barriers to implementation. This theme is composed of two sub-themes: 1) time and energy costs and savings, and 2) economic costs and savings. The second theme identified was *Control and Decision-making*. This theme included the increased sense of control farmers gained through their use of technology and the ways this affected their relationships with their animals. This theme included the limits of this additional control that became obvious through technical failures or lack of trust in technology. The third theme, titled *Governmental Influences and Pressures*, included the various social influences, such as regulations and subsidies, applied by the government to impact technology adoption. This included how this social influence was received by farmers. *Out with the Old and In with the New*, which was the fourth theme, discussed the beliefs held by farmers about age and its links to technology. It tackled the dichotomy between "old-fashioned" farming and technological farming. The fifth theme was titled *Curiosity and Excitement*. This theme explored the fun and exciting aspect of PLF technology.

6.3.2.1 Resources and savings

i. Time and Energy Costs and Savings

The main drivers behind technology adoption for our participants were the savings they hoped to experience. These mostly consisted of a reduction in the amount of time and energy the farmers needed to spend on daily tasks and management activities. This theme was identified through many farmers using the expression "time is money," for example. Overall, the farmers appeared to be looking to improve the efficiency of their work on farm.

> **Farmer P98:** "It's saves us a lot of time."
> **Interviewer: "**and money?"
> **Farmer P98:** [laughs] "it's getting money when you save time."

Many farmers were happy to pay the price for technology to ease the physical and mental toll that work on a sheep farm can involve.

> **Farmer Q34**: "… even if I have a high capacity, it's limited. Yeah. At some point. Yeah. So I like to work with the sheep. But it comes also with a cost. Yeah. So the time and work and energy I use there. I doesn't... I can't use somewhere else. I can't use all... sometimes it lowers my battery. And so [with technology] I have more energy to... but to a certain degree. A day like this up in the mountains, you are tired when you come home. For sure. So tomorrow, I I Maybe can't work 10 hours, I have to just work five hours."

> **Interviewer:** "okay and what made you decide to pay the money to get it?"
> **Farmer C28:** "My arms! He [husband and farm co-owner] worked away, I was much alone."

Some farmers also noted how technology could reduce cognitive and emotional burdens, for example reducing confrontations with neighbours thanks to digital fencing, reducing anxiety over leaving the farm for holidays by providing sheep location information through GPS, or helping keep the sheep organised in age groups without any paperwork or memorization thanks to an RFID recognition system.

> **Farmer Q34:** “It's sorted there. So I just, when I go up [in the barn] and, and are doing the work. It's already… the brain work are done. It's just- so when you're standing with the dog and the sheep and it get messy…”
> **Interviewer: “**Yeah, for sure.”
> **Farmer Q34: “**So I can do that. I love this.”

When their expectations of time and energy savings were not met and the cost was not outweighed by the benefits, several farmers reported a lack of trust in the technology company, which resulted in indefinite termination of product use. According to the farmers, it was not possible to win back this trust once it was lost.

> **Farmer I58:** “because we used to have [Company A]. But they went, they company went bankrupt and we didn't trust them anymore. So we stopped using it. We had 47 of those. […] It worked very good for the first year. But then they had some problems and couldn't give any support or anything. So it was just very bad. And people have bought many items on it! Yes. So. […] This is the same people so we don't trust them. [all laugh]. It cost too much too. So I think they tried to make a connection between mother and lambs. But we haven't try that.”

Additionally, there were often hidden time and energy costs, such as when unforeseen work was involved in setting up, updating, or maintaining the technological tools (e.g. programming the signal frequency of GPS collars or updating software). This issue degraded the perceptions of value and savings, primarily because these costs were unexpected.

> **Farmer W37:** “And you see, this is two batteries and two plugs. And when you have 50, 50 pieces of… this is a big job. Put them back in with the screw, testing that it works. See it's on the app. And everything. It's, it's not, it's not quick fix? Yeah. No change in my, just one battery. That's more. Okay. And this will last for four more years. This will have to change every year.”
> **Interviewer: “**Ah, okay. Yeah.”
> **Farmer W37: “**And battery. Of course we have to change after season. It's about in

this time. Yes, I would think between 20 to… 20% defect problems. Okay. And it's not better. It's not less problem. That's, that's strange."

Despite investing in technology to save time and energy, many farmers still felt like these resources were limited. This concern materialised in the context that farmers were aware that the tools they bought had more functionalities than they were actually using. Thus, a lack of time or mental energy to learn acted as a barrier to using PLF to its full capacity. These farmers stated that they were satisfied with their use of technology and could not afford to put in more time or energy to learn the additional ways they could be using it. This suggests that despite PLF helping with time and energy savings, these resources will always be in short supply on farms.

Farmer T30: "And you can also look for if she had good big lambs, you can see where she is going and you know there was good grazing, we can use it in a lot of ways, but it's the time that you need some time to look for it. And I do not have it. Yeah."

ii. Economic Costs and Savings

The technology also impacted farmers' finances, which was another important driver of adoption. The time and energy savings allowed some farmers to pursue other types of work which provided additional income. Although few farmers had quantified the financial gain, it was nonetheless their perception that PLF had benefitted them financially. The few farmers that had carried out cost-benefit assessments reported that technology was crucial to creating this additional income.

Interviewer: "Do you feel it saves you any money?"
Farmer L61: "It kind of does. Because if I have more time down here, I can do some... It's not that good paid with the sheep. So and if I have some days more, I can stay at home and do other work, whereas more income, so yes, it does save me some money. But I can't put numbers on it."

Interviewer: "So. Okay, so the technology saves you time and energy. Do you feel that it saves you any money?"
Farmer Q34: "Yeah, because I do other jobs too. And every hour spent on the sheep cost me..."
Interviewer: "Money you could be making the other job. Yeah."

Farmer Q34: "So every time every hour spared, I'm earning 550 krones. Okay. Plus, tax."

Thanks to the technology, farmers who owned automatic feeders or feed mixers said that they saw an increase in their lamb weights, and therefore in their profits, compared to past years. However, none had calculated whether the cost of technological tools had been paid off by this increase in meat profit.

Farmer R64: "So now, we are delivering, now really going to come out at 23.5 kilos in meat."
Interviewer: "Because yeah….average."
Farmer R64: "So that is very good. It's the biggest in my time. That's great. Yes."

Some farmers specified that given the technology's costs, they expected durability and consistency. Durability was defined as every component resisting the rough, dirty, and wet conditions on farm over significant periods of time. Consistency referred to the farmers' desire for a constant, seamless delivery of services over several years, without regular changes or updates to the system.

Farmer F50: "The least favourite thing about the collars is that they stopped working. Because the technology is having a rough time around the neck of sheep. So they are not very sustainable."

Farmer I58: "Yeah, but you have to have a system, you have to have the […] PC computer programmes and they change it. [Company B] change it several times so… and then I have to do something new. Yes, it's annoying because we have - in May it's very busy. So it [the software] has to be there."

Several farmers were happy to incur the high cost of technology because it ensured the family farm would be taken over by the next generation. Integrating technology was seen as keeping young people interested and invested in farming, while allowing them to pursue other revenue streams with the time it saved. This is illustrated in the following extract, where a farmer describes his hope for the future of their family farm now that he has invested in an automatic feeder and a new barn.

> **Farmer R63**: "I think when my son has taken over, when he is feeding in the morning, he can just touch button and then they eat and [he can] go to work. When I work, I like to look at the sheep."

6.3.2.2 Control and decision-making

Most participants expressed added control as one of the reasons why they liked and used technology. They felt the extra information provided about their sheep, such as location, weight, or family history, enabled them to make informed decisions about their business, increasing their sense of control over their success.

> **Farmer E99:** "This is probably the main, the main thing for sheep farmers: to have control. Where are my sheeps (sic) and they don't escape. I must admit that it gives very good digital [information]."

When asked about their relationship with their sheep, many farmers felt this increased control improved it. Knowing more about their sheep improved their ability to care for them, both as individuals and as a flock. This perception of increased knowledge and care led to the farmers feeling closer, or more connected to their sheep. As illustrated in the following extract, it is clear that for many farmers, control and a good relationship with their sheep were linked.

> **Interviewer: "**And since you started using the scanning and the RFID, do you think your relationship with your sheep has changed?"
> **Farmer I23: "**Maybe, I think to the better because I have better control with my sheep."

A few farmers acknowledged that a possible negative spillover effect that came with increased control is a disconnect between farmers and animals. However, these claims were more anecdotal such that these farmers stated this was not their personal experience but rather the experience of other farmers. These same farmers stated that their perceived responsibility to the animals meant that they would not permit PLF to distance them from their animals.

> **Farmer C28: "**I know [some]one who got the camera in her house. Yeah, when lambing lambs comes in spring, she sits in and driving the camera around and see Oh, that's good. It's good. She wasn't in in in your barn."
> **Interviewer: "**Yeah. In the barn."

Farmer C28: "Yeah, I, I, no, no, I don't like that. You want to be I want to be there. And if it is something wrong, you had to stay there and observe through all time and get in when when you've got help. And then you can save a lot of animals."

Predator attacks were often cited as situations where technology could increase farmers' control. Tools such as GPS collars were identified as being particularly useful in identifying predator attacks. The farmers noted that this tool can flag unexpected movement patterns of sheep (e.g. dispersing, running many kilometres). They concluded that these metrics indicate a likely predator attack or observation of a predator by the flock. However, beyond being aware that an attack is occurring, there was little evidence that GPS collars could prevent predator losses or improve the outcome of attacks. Some farmers reported using location data to make breeding decision to protect their flock against future predators by encourage tight flock structures. Based on the GPS maps, they bred from ewes that stayed close together in safe areas and did not breed from or culled ewes that separated from the flock often.

Farmer C42: "We can also see if there, already if the flock is spreading, you can see it: the predators. So it's, that's good"

Farmer F50: "So then I get the clue of when they are leaving and if the lambs are worth having as as ewes because if they walk, in June then they walk away directly out of the grazing area and the lambs are not interesting for for breeding. But if they are walking in late August and first of, the first week of September then they have been all summer in the, in the area so, they they would probably be hefted to this area to use in English expression."

There were limits to the added sense of control brought on by technology. One such limitation was where farmers did not trust the technology to make accurate measurements. Believing the tools are reliable was crucial to an increased sense of control. If there was no trust in the technology, then using it would bring on fearful or sceptical beliefs.

Farmer Q61: "They are many farmers are afraid of the grain feeding system, they'll think it's too expensive and too advanced and really difficult."
Interviewer: "So, yeah, when you say, you said that before too: that this grain feeding

system is scary. They're afraid of it? Like what, what is it that they're afraid of?"

Farmer Q61: "Computers a bit? Okay, everything with a computer in it is dangerous."

Interviewer: "So they just don't feel confident using the computer or they don't trust the computer?"

Farmer Q61: "It's, I think it's a mix of many things. But many farmers are practical guys. They want to use a hammer or a bench or a saw. Something like that. And if you use that to the computer, that's not going to.. ever..."

Additionally, one farmer pointed out that all the additional information can result in added anxiety, rather than control. The pressure of always being connected weighed on them and actually decreased the feelings of control. Interestingly, this participant had said earlier that technology increased the control they held over their operation.

Farmer B42: "But in a way you when you have all this information, you also feel you're never free. Just Oh no. It can come in at nighttime or 11 in the evening."

6.3.2.3 Governmental Influences and Pressures

Many farmers identified different levels of government (municipal, regional, or national) as facilitators in acquiring and utilising PLF technology, while some saw the government as a hindrance. There was no consensus on the government's role in helping farmers use PLF. Those who received subsidies from their municipal or county administrations felt supported, while others thought the government reduced farmers' access to technology by creating too much red tape.

Farmer W37: "Yeah. I say that because this this community buying this equipment getting at least 50% of cost by this cost? No, I think 1000 Norwegian kroner or something? Each. And I think the government scheme and…they have been very nice to our area."

A couple of farmers stated that the government's support of technology was strictly linked to their hope of reducing predation on sheep flocks, therefore reducing the payments made to the owners of sheep killed by predators.

Interviewer: "Is there any other ways that the government is encouraging Norwegian farmers to use technology?"

Farmer F50: "Not so much. It's, it's mostly that and that's because of the predator problem. Because of the growing presence, problem they have. They have a[n] organisation of a system that is called organised grazing. And they have some conflict, prohibiting things to that, they, they use money for. Okay. And that's among them. That's so it's, it's a special budget that the government has to keep the conflict with the predator and the farmers [under control].”

Farmers remarked that the government played a role in their use of technology not only through financial incentives, but also by exerting social influence. Some felt there was social pressure being applied to “push towards more technology.”

Interviewer: “do you think the Norwegian government encourages sheep farmers to use technology?”
Farmer Q61: “No, I don't think so. There's a big discussion going on between farmers that if you're going to get your subsidies, you should have this sheep control system [registration software]. Okay. That's the point, the opinion of some farmers, of course, they are always using the system. And I think we get a lot of money from the government. And I think we all have to prove that we are worth all this money. So that to force the sheep farmers to use, for example, the sheep control system. I think it's a right thing to do. But it's going to make a lot of noise.”

A good example of the government using its influence to promote PLF is their consistent support for a company I will refer to as “Company C”, founded in Norway. Many participants cited them as being a popular PLF tool in the country. They were aware of the government’s support of Company C’s business development through subsidies and networking opportunities. They also felt they had received messaging through the government to purchase Company C’s products, given that grazing associations received subsidies to purchase Company C's collars.

Farmer L61: “They did with [Company C]. That I know. Because [Company C] when they started there, it was quite difficult to get everything right. And there was the government pushing it, that they would keep working on it. They just said we have to have that one, that technology we need.”

Interviewer: “And do you think that the Norwegian government supports sheep farmers who use technology?”
Farmer I23: “Yeah, they do. Yeah. Yeah. Because I know the people in [Company B]. They are in Africa. Now they use it on... in the national parks in Africa. And I know that the woman with the government has been with [Company B] to Africa for a week last

winter to help them and see how they can make them connections. […] I know the, the boss of [Company B], he was with the king of Norway for dinner."

6.3.2.4 Out with the old and in with the new

A theme around age was identified. There seemed to be an incompatibility in participants' minds between technology and older people. Some farmers also reported perceived ageism. For example, in the extract below a farmer claimed to be unable to access government funding for the construction of a new barn because the subsidies were set aside for young farmers and he was deemed to not meet the age criteria.

Farmer R63: "No, no. I didn't got [money], because they [the government] said… it's in 2017-16, there there was too much sheep in Norway. And it was difficult to get some money. So they said told me I'm also old. Okay, yes…"
Interviewer: "And that's why...?"
Farmer R63: "They say, when my son has taken over the farm, then he can get almost a million krones with a build."

Other age-related references were identified from those that held the belief that youth was implicitly linked to technology use.

Farmer T30: "Yeah the young people they like much technology. I think they like it, it's… to work with it. Yeah old people are more... doesn't matter. Yeah."
Interviewer: "So was it difficult to learn how to use it?"
Farmer T30: "I have some good helpers. Okay. The young people." […]
Interviewer: "And is there anyone else that works with the sheep here? Is it just you?"
Farmer T30: "I have one what you call it? […] one one worker. And in full time and I have some seasonal help. Yes.
Interviewer: "And do they work with the technology as well? Or it's mostly ..." […]
Farmer T30: "yeah, they do it too. they are good, more good to that than me. But I decide."

There was also respect shown towards the older generations of farmers and their way of doing things, referred to as "the old days" or "the old-fashioned way." But it was often combined with a sense that it conflicted with a PLF approach.

> **Farmer Q61:** "Yeah, I think I'm going to, back to the old-fashioned way to deal with the feeding."

> **Farmer Q61:** "Because in the old days, I have 100 sheeps (sic), and they were the same. But I started to get the system made me pick out which one is producing and which one is not. And that was a good thing. And my father was always doing the old fashioned way. Oh, this looks good. This was good last year.. yeah, the farmer senses."

Some farmers referred to themselves as the "older generation" and acknowledged that their way of doing things would not continue. They expected their sons and daughters to use more technology and to stop using some of their parents' methods. As mentioned in the Resources and Savings theme (6.3.2.1), these older farmers had often invested in technology for the sole purpose of securing their children's commitment to taking over the family farm.

> **Farmer F50:** "I think probably it will increase. Yeah, I think when my son takes over in a couple of years, I think he will. He has been growing up with technology. He's now 29 on this. He has been on the computer since he started school, so he is he is so familiar with it that it will naturally take. Be more. I'm from the old paper generation and I was so I, I doubled books, everything. Still, when I when I'm registering the mating or the lambing, then I have a handwritten list. And the computer. Yeah, because I don't trust the computer completely. He will probably just use the computer. He won't have this book that is noting everything."

However, it was acknowledged that not all young people are necessarily interested in using more technology, describing some young farmers as "more conservative" despite their age. It was suggested that more conservative or traditional views in younger farmers stemmed from how they were taught to farm and their belief in the more traditional ways. For example, one farmer described a neighbouring farm where the young son who was due to take over the family farm did not place value on technology because his father had taught him to raise sheep "the old-fashioned way." Participants also discussed how technology has specific benefits for older farmers and is therefore of benefit to all age groups. For example, auto-draft machines and automatic feeders could reduce manual labour, protecting older farmers' physical health and increasing their longevity in the workforce.

> **Farmer I58:** “I think this weight [crate] which we have bought, it will save our body because this sheep will learn to go in a row and into the weight. And we will "beep" in computer and we don't have to do all the with our arms and shoulders and it will save our… I think it will be good for us. Yeah. And that's important because...”
> **Interviewer: “**Yes, the longevity of your work.”
> **Farmer I58:** “Yes, and we are not getting younger. But for younger farmer it will be better than that. They can live longer. Yes. It is very important to think about health. Many farmers are very tired when they're getting 60 and that is too early. Because they are not old. Definitely so, I think technology is good for us to help us and we will use it if we can. Yes we are not against it. We are very pro and I think maybe younger people think that farming can be interesting with technology they are used to use it. We use all these programmes at computer it is registration and all these things. And that is helpful for us too.”

Participants often mention the special interest older family members and friends took in the technology and the new information it can offer. With fewer farming responsibilities of their own or having changed industries completely, they were intrigued by the reports generated by the technology.

> **Farmer C42**: “Even my father! Because he's old. And also old, but he's he's most interested because in the app and see where the sheeps (sic) are and just: oh, she's gone there and she's gone there”.

> **Interviewer: “**have you ever done that? Have you ever showed your app to someone who's not a farmer?”
> **Farmer I69**: “Yeah, many times. Yeah.”
> **Interviewer: “**What kind of reaction do you get?
> **Farmer I69**: “They say, yeah, oh damn! and they think it's funny to see. Yeah. Many of people a bit older than me, all of them have probably been collecting sheep at one time when they were young, and they went with their grandparents had a farm. So they remember that then.”

Finally, this view that farming is an “old” profession and incompatible with technology seems to be seeping in from the urban public, who can think of farmers as backwards country people, cut-off from the world. But some of the participants identified their use of technology as an opportunity to challenge this view and share their high-tech reality with the public.

> **Farmer T69:** "Yes and no, because they are... people who don't know what we are doing either they think we have a huge fancy tractors and spend a lot of money on that and we complain we don't have money or they see us as old [Norwegian word]. What's that in...?"
>
> **Interviewer: "**country people?"
>
> **Farmer T69:** "Yes. Yeah. But the if I it's always a conversation starter to show them where the sheep are in the mountain. I think people are interested in, to see that. That we are keeping track of the sheep and know where they are okay. And I think it's very important."

6.3.2.5 Curiosity and excitement

The final theme identified from the reflexive thematic analysis of the interview transcripts was the simple interest farmers had in learning more about their sheep, beyond any practicalities. Farmers found it fun and exciting to be able to see where their sheep were, despite being miles up a mountain, for example. They enjoyed sharing this information with others, i.e., farmers and non-farmers. As mentioned when describing the theme Out with the Old and In with the New (6.3.2.4), several participants referred to the special interest older family members – now with fewer farming responsibilities – had in following the reports generated by the technology.

> **Farmer I69: "**I didn't...I didn't believe they walked those distances every day. As they do. They move a lot more around that I think. And now I know where they sleep at night. So it's, it's funny to see."

> **Farmer C42**: "Even my father! Because he's old. And also old, but he's he's most interested because in the app and see where the sheeps (sic) are and just: oh, she's gone there and she's gone there".

Often, the excitement elicited by the technology led farmers to think about what could be possible in the future using technology. They described yet to be invented or commercialised tools that would help them or that they would find interesting. Many thought that tools to detect disease, oestrus and labour would be most helpful, while others mentioned localisation devices that could read the distance between ewes and lambs. In general, there was interest in technology for lambs, since most commercially available tools are only suited for adult sheep.

> **Farmer T47**: "…some people are trying to, yeah they have these ear tags, they put some... So you get some information related to [body]heat temperatures and so on. That I think that will come in. Sometimes in the future maybe that's good for farmer, it will be positive for detecting diseases. I know you can also find out if they are stressed but that would be mostly positive."

> **Farmer F50:** "So it would probably be some, just some years if I had the new, perhaps have the communication between the mother and the lambs and so on. So we know that the because these collars we put on the ewes, but the wolverine, he takes the lambs. Yeah, exactly. So we don't know if there are any lambs missing. But if you have this communication between the mother and the lamb then we know this mother has lost her lamb, then we can check on."

6.4 Discussion

These interviews have highlighted sheep farmers' multifaceted motivations, perceptions, and beliefs around PLF. I purposely spoke only to farmers currently using PLF, because I was seeking informed opinions on its use. Although their beliefs are interesting and important to a holistic understanding of PLF, I did not interview other stakeholders, such as veterinarians or policy makers, as I aimed to report solely on farmers' perspectives. Speaking to farmers from various regions of Norway allowed me to gain an understanding of any differences and commonalities across geography in this specific country. This explorative study contributed to the evidence on PLF adoption and use in livestock farmers. Our conversations revealed that their motivations were not solely economic. Farmers reported seeking to increase their control and to satisfy a curiosity. The government and farmer age were perceived influences on PLF uptake.

6.4.1 Resources and Savings

As reported in the results, time, mental and physical energy are precious resources for farmers. The utility of PLF to reduce the expenditure of physical and mental resources proved invaluable for some farmers. Saving time was the reason most often mentioned by farmers. Indeed, time-saving is one of the principal objectives of technology in most contexts, including agriculture (Bonabana-Wabbi, 2002; Mwangi & Kariuki, 2015). Saving time and energy are a major part of the performance expectations that farmers have when adopting technology (Devitt, 2018). Naturally, saving physical energy is important as farm work is physically arduous and farmers are at increased risk of musculoskeletal disorders and injury (Walker-Bone & Palmer, 2002).

I also found that PLF was beneficial in reducing cognitive and emotional burdens on farmers, such as reducing anxiety and the burdens placed on memory (e.g., having to remember pedigree information or weights). Research from across the world has shown that farmers suffer from navigating the cognitive load (e.g. data management, decision-making) of their work mostly alone, and face stigma and a lack of support services if this isolation leads to mental health challenges (Deffontaines, 2014; Eastwood et al., 2023; Fuller et al., 2000; Lunner Kolstrup et al., 2013). In fact, farming is listed as one of the 10 most stressful jobs in the USA (Sauter et al., 1999). The introduction of PLF also brings a new set of issues, such as the steep learning curve involved in learning PLF functions as well as the handling of the huge amounts of data produced by the technology (Eastwood et al., 2023; Nazareno & Schiff, 2021). However, in line with our findings, past research has found technology can also free more time to spend on other tasks, increasing connection with animals and other staff, helping with decision-making and reducing the cognitive load of farmers (Eastwood et al., 2023; Hostiou et al., 2023; Nazareno & Schiff, 2021). This echoes our finding that technology freed up time to watch the sheep, which increased the farmers' feeling of connection with them. Our participants similarly reported feeling more confident in their decision-making thanks to PLF. Indeed, a reduction of the cognitive load can be a driver for uptake of technology.

It is of course expected that farmers will consider cost when making decisions about technology. This is especially true of sheep farmers, who have been identified in the UK as having the lowest profit margins of all livestock enterprises (DEFRA, 2014; Kaler & Ruston, 2019). In Norway, sheep farmers made similar profits as beef farmers, but both made much less than pig farmers or beef farmers who also produced cereals (Knutsen, 2020). Since 2017, the Norwegian sheep population has declined by 12% and many farms have gone out of business (Knutsen, 2020). The farmers that remain have been faced with reduced profitability (Knutsen, 2020), most likely due to rising costs of materials and labour combing with reduced demand for lamb meat. In this financial context, it is not surprising that farmers investing in technology are motivated by increasing their productivity. In a case study of EID uptake as a management tool in British farmers, 66% of farmers agreed or strongly agreed with the statement "The cost of equipment is important to my decision to use EID recording for farm management" (Lima et al., 2018). Interestingly, the farmers in our study did not factor cost into their decision around PLF purchase. This finding aligns with past research that reported that farmers report knowing little about financial gains and profits (Kaler & Green, 2013). It was suggested this was due to a lack of record-keeping, which the farmers did not like to do and therefore did not prioritise (Kaler & Green, 2013). A survey of English and Welsh farmers similarly found that 62% of respondents had problems with record-keeping and paperwork (Simkin et al., 1998). It is possible that not examining their farming practice through an economic lens is a reaction to the low level of control held by farmers over their circumstances. It may be a defence mechanism in the face of the large impact external forces such as weather, fluctuating markets and government regulations can have on their profits (Lunner Kolstrup et al., 2013), in the sense that by not tracking costs, they cannot be disappointed when an external force negatively impacts them. Or it may be that their motivation for farming was never financial, and therefore keeping detailed records of their finances is not a priority to them. In the author's experience, farmers are driven by local culture, family history and personal interest, so they may regard PLF through those lenses rather than an economic one.

In the often-challenging farm environment, whether it be the outdoor grazing areas or the indoor barn system, durability of products is required and prioritised by farmers. For example, 66% of combine harvester owners answered that access to service (and therefore machinery longevity) was a decisive factor in their purchasing decisions (Eikel & Rademacher, 2001). It is therefore not surprising that the participants in this study expressed frustration when constant troubleshooting or updating was needed for the technology to perform as expected.

The concern that PLF development will occur at the cost of farmers' time and money has previously been highlighted in the literature (Wathes et al., 2008). Authors have raised the question of whether sufficient market research is being done by engineers to truly meet farmers' needs (Wathes et al., 2008). Some of the discourse collected for this study suggest that products are in fact still being developed at the farmer's expense. If the products do not then deliver on their promises, farmers lose trust in these companies. Conversely, a survey of broiler, pig and dairy farmers found that farmers receiving sufficient support from the providers held a positive view towards the PLF systems (Hartung et al., 2017). This finding was reaffirmed in the present study i.e., farmers who were satisfied with the level of support they received from the PLF companies were equally satisfied with the tools. In these circumstances, trust is linked to the degree of confidence, predictability, faith, or cooperation that prevails in the provider-user relationship (Kasperson et al., 1992). I saw this through many farmers mentioning Company A having gone bankrupt and their refusal to engage with the company even though it is back on its feet under another name.

Some farmers also recognised that they were not using their PLF tools to their full potential. They reported a lack of time to learn the different functionalities as the main reason for this. European livestock farmers are constantly under a long list of pressures (Hartung et al., 2017). Not only do they face significant and mounting economic pressure, but they must answer societal demands, notably: providing food security, food safety, food affordability, environmental protection and animal welfare (Hartung et al., 2017). When the work of all participants on a livestock farm is considered, the average work time is equivalent to 581 days per year (Sraïri & Ghabiyel, 2017). It is then no surprise that farmers feel they do not have time to fully explore the potential of PLF, even if it promises to alleviate some of these pressures. As has been noted in previous studies of technology adoption, strictly economic models cannot account for the complexity of farmers' motivations and behaviours (Flett et al., 2004).

6.4.2 Control and Decision-making

Research on British sheep farmers found that tools that empower farmers to be in control and that do not affect the human-animal relationship might encourage uptake of PLF technologies (Kaler & Ruston, 2019). Similarly, a study of Norwegian farmers' adoption of Internet of Things technology reported that increased control was one of its perceived advantages by farmers (Lillestrøm, 2021). Participants in our study also discussed how the technology increased their control and benefitted their relationship with the sheep. This perceived improvement in the human-animal relationship was interesting, as many studies of PLF have highlighted the deleterious effects on the human-animal relationship it could have (Buller et al., 2020; Cornou, 2009). Most farmers interviewed in our study seemed aware of this risk but did not feel it applied to them because contact with their sheep was a priority. Others have also reported farmers taking action to ensure the technology does not impact their relationship with their animals (Hartung et al., 2017). In the current study, our farmers provided several explanations for safeguarding and prioritising their relationship with their sheep. One reason was that some thought having a good relationship with their animals reduced sheep stress at handling, while others explicitly stated it is impossible to be a good farmer while not being in contact with your animals. Some farmers simply enjoyed taking the time to observe their sheep, which does not contribute to building a two-way human-animal relationship but did lead to farmers feeling of increased connection with their flock. The literature on human-animal interactions and their impact on animal welfare tends to agree with these farmers' points. Sheep can recognize regular caretakers' faces and remember them for up to 2 years (Kendrick et al., 2001; Knolle et al., 2017). This suggests that regular contact can help create a positive human-animal relationship (Rault et al., 2020).

The view on the role technology plays in managing predators was shared by almost every farmer interviewed. Despite no participants being able to take direct action on predator attacks through technological data, they all felt that it was still helpful in indirect ways. For example, they could use GPS collars to select ewes that stayed close to the flock to breed from, therefore weeding out ewes that roamed and were more susceptible to predator attacks. However, very few farmers applied this idea in practice. When they spoke about technology and predators, it was mostly hypothetical, meaning they had many ideas about its potential for reducing the impact of predators, but none were applied on their farms. This interest in using technology to manage predators may reflect interest from the research and policy worlds. Many studies model or simulate predator attacks to extract technological data that could identify real attacks (Manning et al., 2014; Virgilio et al., 2018). In 2004 and 2011, the Norwegian parliament came to two decisions, referred to by the public as the Carnivore Settlements. These settlements aimed to reconcile continued sustainable livestock production and the maintenance of viable carnivore populations (Strand, 2021). There is no doubt that sheep farmers have therefore been inundated with media and government messaging about predators over the last 20 years. In fact, a few participants commented that this was a government priority. As such, although there is a shared agreement around the potential for PLF technology to help manage predators, current and practical applications are scarce.

The farmers described the limitations on how much control they could gain from using PLF tools. These were the same frustrations they expressed when talking about their expectations related to cost. As described in the Resources and Savings theme, troubleshooting and lengthy set-ups created additional work for users that took more time rather than saving them time, as promised. This could contribute to decreased trust in the product and a decreased or unchanged sense of control. Most farmers interviewed were enthusiastic adopters of technology, but they spoke about others who do not trust that technology can make more accurate measurements than humans. Looking at mistrust through a Theory of Planned Behaviour (TPC) lens would suggest that farmers who have a negative attitude towards PLF are much less likely to adopt PLF or show intention to adopt it compared to those who have a more positive attitude (Ajzen, 1991). Applied in a range of disciplines, TPC outlines that intention to perform behaviours can be accurately predicted by attitudes towards the behaviour, subjective norms and perception of behavioural control (Ajzen, 1991; Morris & Venkatesh, 2000). Research suggests that distrust in technology acts as an inhibitor of acceptance (Parasuraman, 2000). For example, EID adoption as a management tool among farmers was affected by level of readiness as measured by the Technology Readiness Index (TRI) (Lima et al., 2018). The TRI is a standardised measure of consumer readiness to embrace new technologies (Lima et al., 2018). To increase trust, one must increase the farmers knowledge and understanding of the technology, suggesting that to increase motivation and intention one must remove knowledge and skill barriers (Lima et al., 2018).

6.4.3 Governmental Influences and Pressures

Several farmers discussed governmental actions and regulations as external forces that impacted their practices. A mix of optimism and scepticism was recorded when farmers expressed their opinions on the role of the Norwegian government in PLF adoption. Some participants benefitted from subsidies through farm grazing groups, sometimes covering up to 90% of the costs of technology. Others felt the government cost them more money through bureaucracy or not fitting the criteria for subsidy eligibility. As a whole, Norwegian agriculture relies heavily on governmental subsidies due to small farming units and high costs (Lundekvam et al., 2003). In 2020, Norway deployed the Food Nation Norway Initiative, a political framework aimed at increasing and promoting sustainable food production. Two stated goals of the framework were to “Develop a strong supply-sector of technologies and services” and “Support the development of comprehensive innovation systems in the area of food that can respond to societal needs.” These aims explicitly state that the government will use its social influence to support technology in agriculture. However, this display of support was seen as a social pressure by some farmers who felt pushed into using technology to continue accessing subsidies, for example. From the shared experiences identified in this study, it seems that to align with the goals of the Food Nation Norway Initiative, the government made funding available for sheep and cattle farm grazing groups (rather than individual farmers) to purchase technology and directly supported the creators of livestock technology through networking events and subsidies.

6.4.4 Out with the Old and In with the New

According to some participants, advanced age and technology could be incompatible. Using PLF appeared inextricably linked to youth, with farmers of all ages reporting that young people were more interested in and adept with technology. One of the questions asked in the interviews was about the participant’s age. It may be that this question, the significant age gap between the interviewer and the participants and the context of the interview, i.e. speaking about technology, triggered a focus on age. I therefore wish to recognise and emphasise the contextual influences on this theme. The differences in age between the interviewer and the participants perhaps provided us with the opportunity to identify different types of knowledge for example. As noted, age differences between interviewer and interviewee can refine interactions, shape rapport and influence the data acquired (Vasquez-Tokos, 2017).

Most farmers in the current study linked youth to technology. They either decided to invest in technology to ensure the next generation would be interested in taking over the farm or depended on the young people around them to benefit from their investment. They all said that younger people were more comfortable using technology. Interestingly, a previous study did not find that age affected EID adoption for flock management and highlighted the lack of a clear link between age and technology adoption in the agricultural literature (Lima et al., 2018). Similarly, our participants' age did not affect their PLF adoption. In fact, the second oldest farmer was the one who had purchased the most types of PLF tools and the youngest only used one type. There is some evidence from outside of agriculture that age influences adoption of technology by affecting the level of importance accorded to it when making decisions (Morris & Venkatesh, 2000). Interestingly, this research reported that older workers were more likely to make decisions about adopting a new software system based on the perceived increase in control it offered (Morris & Venkatesh, 2000), echoing some of our study's findings.

On one hand, ruralness and farming is built on respect for seniors and their life experience (Mungmachon, 2012). On the other, the growing role of technology on farms may be eroding the perceived value of older farmers' experience in the eyes of the next generation. The risk that new technologies are likely to further marginalise the most vulnerable people in the industry has been acknowledged in the literature noting that equitable and accessible technology must be prioritised (Rotz et al., 2019).

Some participants had invested in technology to ensure that their children would take over the farm when they retired. Their own use of PLF was limited but they had a vision of the future where it enabled their children to work on the farm and keep another job, securing more streams of income. There is evidence that technology adoption can help revenue enhancement and production efficiency through diversification and off-farm income (Fernandez-Cornejo et al., 2007; Morris et al., 2017). Other farmers took a different approach and very much invested in technology in support of their own physical and mental health into old age, ensuring the longevity of their business in a different way. While there is evidence that technology can replace some of the manual labour required in farming and lighten the physical burden of farmers, it also creates a new task for them, e.g. supervising the tools to ensure they act as desired (Hostiou et al., 2017a; Lunner Kolstrup et al., 2013). Further work is also created when machine breakage occurs, leading to more stress (Lunner Kolstrup et al., 2013)

Farmers' perceptions that they are viewed as out-groups by the public is not new. Evidence from the UK suggests that consumers do not care to reflect on the origin of their food, especially meat, and often hold negative views of farmers (McInerney, 2002; Weatherell et al., 2003). However, there is a new category of consumers emerging who buy local products in search of a different type of connection with farmers, one based on reciprocity, trust, and shared values (Weatherell et al., 2003). Interestingly, the Norwegian public appear very satisfied with Norwegian agriculture, with an alliance between producers and large groups of consumers (Nygård & Storstad, 1998; Storstad, 2001). However, there is very little recent evidence for us to draw upon to know whether these perceptions remain in the Norwegian public today.

6.4.5 Curiosity and Excitement

Finally, another pattern that was identified in the data was farmer comments about how "fun" and "cool" the technology was. When asked what drove them to purchase it, some responded they were simply curious to try it. Many enjoyed sharing the new information they obtained with lay people and others who had little to no responsibility on the farm, such as older relatives. Curiosity is a basic biological driver of humans and has been identified as an important motivation for learning and discovery (Datt et al., 2013). Past research has also found that "experimenting purposefully with curiosity" was a main driver in 60% of animal husbandry innovations by Indian farmers (Baliwada et al., 2018). The study also categorised farmers as innovators or non-innovators with the innovative farmers identified as being more socially empowered (Baliwada et al., 2018). It would be interesting to explore if the curiosity identified in our study was also associated with perceptions of social empowerment. This can be identified from our findings to a certain extent whereby our participants reported increased self-confidence and took actions such as joining a farm grazing group or speaking about their PLF experience at meetings, which can create social connections.

When talking about their hopes for the future of farming technology, farmers focused on early disease detection and location information that considers the ewe and the lamb. Both topics are aligned with current research on PLF technology, meaning that the technology farmers are hoping for may not be far from reality. Examples of early disease detection include the Targeted Selective Treatment (TST) using live weight gain to detect and individually treat gastrointestinal parasites (Greer et al., 2009; Kenyon et al., 2009). The TST concept has successfully been applied to identify lambs requiring anthelmintic treatment (Morgan-Davies et al., 2018). Lameness has also successfully been identified by technology before it is noticeable to the naked eye, although these tools are not currently commercially available for sheep (Barwick et al., 2018; Kaler et al., 2020). The ewe-lamb spatial relationship has been described and maternal pedigree has been identified using Bluetooth beacons (Sohi et al., 2017). Clearly, the topics that evoke excitement and curiosity in farmers have the same effect on researchers and research funders. Little academic research seems to be devoted to understanding what farmers expect or prefer from future technology, despite the abundance of review papers on all the new tools available. Their input should be prioritised in research on future technologies. This study adds to the evidence that curiosity is an important driver of problem-solving on farm (Baliwada et al., 2018). As such, it should be encouraged and supported in farmers.

6.5 Conclusion

The five themes identified in interviews with Norwegian sheep farmers about PLF were the impact of cost, the increase in control, the influence of the government, the relationship between old age and technology, and the drive of curiosity. A key finding was that farmers' motivations when using technology are not entirely economic, although time costs did play in a significant role in the decisions they made around technology. Farmers were satisfied with their PLF tools when they felt they increased their control and improved their relationship with the sheep. Dissatisfaction occurred when they did not ease the mental and physical workload. There were varying opinions on the role the government played in supporting farmers using PLF, meaning the roll-out of any initiatives has likely been patchy. People often automatically linked young people to technology, although no such pattern arose from the data. Therefore, it should never be assumed that only younger users will be interested in new technology. These findings could be helpful to developers of future PLF products or researchers looking to test the feasibility of new technology. Despite farmers being unable to share concrete evidence that PLF directly prevented predator attacks, they expressed hope that this could one day be a feature. If this remains one of the main goals of PLF developers, a change in approach or further testing may be required. Finally, many farmers appreciated the exciting nature of gaining new information through technology as it satisfied their curiosity. This aspect should be highlighted when new technologies are being developed or tested: curious farmers will likely be the first users, and they can act as ambassadors for the product going forward if PLF feels fun and exciting to them.

6.6 Supplementary Materials

6.6.1 Topic guide used for semi-structured interviews.

“How do Norwegian small ruminant farmers perceive and use Precision Livestock Farming (PLF) technologies?”: A Topic Guide for Semi-structured interviews. (based on Schillings et al. 2023 topic guide structure)

I. Introduction

• Research topic – describe my experience and research, and what led me here
• Aims – To start to understand how Norwegian farms think about and use PLF technology (what you like and don’t like about it, how you apply it on farm, etc.)
• Confidentiality reminder
• Recording and length of the interview
• Check for questions prior to start

Name:

Region:

breeding females:

Hectares of land:

Type of farm: sheep / goats / mixed (describe) / other (Describe)

Age:

II. General technology use

1) How do you use technology in the day to day operations of your farm?
    a. Which technologies do you use and why?
    b. How long have you been using it?
    c. Why did you start using it? (catalyst)
    d. Seasonal differences in use? Housing vs outside

III. Advantages and Disadvantages of PLF

1) Has using the tech changed your work?
    a. Was there a steep learning curve?
    b. Can everyone who works on your farm use it?
    c. Do you feel you save time and/or money?

d. Do you feel you are getting your money's worth for the tech? Do you feel the investment was fair?

e. What is your most and least favourite thing about it?

IV. Technology across the farming industry

1) How do you feel about using technology?
2) What perception do you think consumers have of technology in farming?
3) How do other farmers in your network use technology?
4) How (if at all) has the government/industry supported your implementation of tech on farm?
5) Do you think it has changed your relationship with your animals at all?
6) Farmer’s own thoughts/Ethics/philosophy on using tech?

V. Looking forward

7) In the future, would you like to use more or less or the same amount of technology as you do now?
8) Do you think there are other areas of sheep/goat farming tech would be helpful in?

VI. Conclusion

Is there anything we haven’t talked about that you’d like to add?

Aims: Reminding confidentiality and other aspects

1) Thanks

2) Confidentiality

3) Contact reminder

6.6.2 Theme Definitions

Theme Title	Theme definition	# of extracts
Resources and Savings	Separated into two sub-themes: 1) Time and energy costs and savings, which covers the spending and saving of farmer time and physical and mental energy as a result of using PLF. 2) Economic costs and savings, covers the financial motivations for farmers to use technology and the monetary barriers to implementing more technology, or any at all.	226
Control and Decision-making	Covers the new information farmers obtain through technology, increasing their sense of control over their flock and affecting their relationship with their animals. Also discusses the elements of farming that remain out of their control due to technical failures and lack of trust in technology.	125
Governmental Influences and Pressures	Describes the patterns of social influence exerted by the government at any level (municipal, regional, national) on farmers' use of PLF. This includes social pressures but also offers of support.	45
Out with the Old and in with the New	Describes the pattern of linking technology to age. Explores the fact that technology seems incompatible with old age and the realities of older farmer's use of technology, or lack thereof.	37
Curiosity and Excitement	Covers comments regarding the fun and excitement provided by the PLF information, as well as where the fun stops and the reality begins.	68

Chapter 7. General Discussion

7.1 Key Findings

In this chapter, I will discuss the broader implications of the work presented in this thesis. I will review how my findings could be useful for welfare assessments and PLF technology development. Specifically, I will discuss how my results could further the understanding of animal welfare and farmer motivations in PLF development. I will also discuss the limitations of my work, and the future research that could stem from it.

PLF approaches are powerful tools for research and farming practice that have potential to improve animal welfare on extensive sheep farms, where shepherds are not continuously near their flock. However, to fulfil its potential, the technology needs to be able to rigorously monitor valid welfare indicators. This thesis aimed to determine which animal-based measures of welfare could be monitored by technology in extensive sheep farming systems, and to test the ability of various tools to measure them. In doing so, it has provided the first steps towards the development of PLF technology that could record informative animal-based welfare indicators. It offers insights into behavioural indicators of welfare, challenges in technology development and sheep farmer perceptions of PLF. These findings have contributed to the growing research on welfare applications of PLF.

Behavioural indicators of welfare were selected due to their well-documented links to sickness behaviour, the difficulty of measuring physiological indicators in extensive settings, and the fact that this thesis was interested in early indicators of disease. The studies in this thesis examined changes in specific behaviours, e.g. a reduction in grazing, rather than comparing activity budgets or studying changes in circadian rhythm. This was because I hypothesised that it would be straightforward to monitor individual behaviours using technology. However, a lot of the data collected for this thesis could be used in future studies taking a more holistic approach to behavioural change. For example, the accelerometers used in Chapter 4 were collecting data 24 hours a day, so links between welfare scores and circadian levels of activity could be investigated. I prioritised identifying indicators of parasitism in Chapter 2 because welfare science evidence of parasite infections causing welfare issues was lacking.

The QBA results demonstrated that parasitised lambs had higher loadings on the dimension describing fear and anxiety compared to one of the two control groups. This may be an evolutionary trait whereby sick sheep are more susceptible to predation, and therefore more fearful. This is one of the first pieces of welfare science evidence to suggest that GI parasitism has an impact on the mental state of lambs. This finding acts as further supporting evidence that early identification and treatment of parasitism should be a priority, so that the duration of negative mental states is reduced to a minimum. Displaying standing inactive behaviour as early as the 14^{th} day of infection onwards was found to be associated with gastrointestinal nematode infection in lambs. This finding was reported in Chapter 2, after running an indoor infection trial where treatment lambs were trickle dosed with *Teladorsagia circumcincta*. It was also echoed in Chapter 3, where lambs naturally infected with *Nematodirus* in outdoor paddocks were more likely to be observed standing inactive as their faecal egg count increased. This increased likelihood of standing was interpreted as an expression of abdominal discomfort as larvae cause damage to the abomasal wall that prevented the lamb from resting and lying comfortably. Lying was negatively correlated with *Nematodirus* FEC in the outdoor trials. Lame lambs were less likely to be recorded grazing than sound lambs and there may have been a cumulative effect of lameness and strongyle parasitism on locomotion and lying in lambs. Lame ewes were more likely to be recorded lying than sounds ewes.

Having identified behavioural parasitism and lameness indicators related to activity in lambs and ewes, the potential for accelerometers to differentiate across sheep behaviours was tested. A validation study was necessary as the AX3 accelerometer had not yet been validated for this purpose. After experimenting with many statistical approaches on the large dataset collected from the accelerometers, k-means clustering was able to partially group the data. Clustering by the mean value of the Y axis could mostly differentiate between lambs grazing, lambs standing, and ewes grazing. It could not identify other behaviours. Identifying lying behaviour proved especially difficult since it was only recorded once during our validation observation period. The lack of lying behaviour in the dataset was disappointing seeing as changes in lying and standing were associated with parasitism in Chapters 2 and 3. The validation study results highlighted the challenges of sensor validation, including manipulating large datasets and obtaining sufficient test observations. However, this thesis was also able to experiment with Bluetooth beacons and readers for measuring ewe-lamb distance, which had previously been validated by Walker *et al.* (2023). These were applied to test the potential of ewe-lamb distance as a welfare indicator around lambing. It was not possible to draw any conclusions about its usefulness as a lamb welfare indicator, but the associations between ewe welfare and distance were analysed. Ewes with fleece problems (e.g. uneven fleeces, fleece loss) were significantly closer to their lambs than ewes without fleece problems. The same was found in lame ewes, whose lambs were closer to them than sound ewes. Though the dynamics behind these results remain unclear, they highlighted an opportunity to measure a potential welfare indicator with PLF technology. This is especially true since ewe-lamb distance was not found to be associated with welfare when estimated during in-person observation in Chapter 3. It may be that ewe-lamb distance only has potential as a welfare indicator when measured precisely and at short intervals, the way BLE beacons are able to.

Finally, Chapter 6 took a social science approach to interview sheep farmers currently using PLF. Norwegian sheep farmers have anecdotally been described as having high uptake of technology compared to sheep farmers in other countries. This was an opportunity to ask current users of PLF about the motivations behind their purchases, their likes and dislikes in the daily use of the tools, and how they see the future of PLF in farming. Farmers were motivated to install the technology in the hopes of saving time and energy. Financial savings were assumed to occur thanks to the time savings. Users of PLF felt an increased sense of control over their flock, which led them to feel closer to their animals and more empowered to make management decisions. The influence of the government over PLF was seen as both positive and negative, with some farmers feeling supported by policies while others felt hindered by bureaucracy. Participants automatically linked technology use with young age, despite being of a broad age range themselves. Older farmers were comfortable assimilating PLF despite saying it was a young person’s tool. Lastly, farmers simply thought it was “fun” and “cool” to gain new information about their flock through technology. It satisfied a curiosity they had that went beyond the practicalities of their daily tasks. This study boiled down the essence of what PLF can be: a tool that saves farmers of all ages and capabilities time and effort, and provides them with a new, exciting look into their flock that increases their perceived control and simply makes them happy.

7.2 Broader Implications

7.2.1 Animal behaviour as a welfare assessment tool

A principal aim of this thesis was to develop valid indicators of welfare that could be measured for a PLF approach to welfare management. Based on a literature review, I determined that behavioural indicators would be best suited to PLF monitoring. This reflects current research on PLF in other species, where for example 82% of PLF tools used in published research on pig welfare measured behavioural rather than physiological indicators (Larsen et al., 2021). Feeding behaviour is often of interest in livestock operations, as seen by the numerous studies testing PLF approaches to monitoring feed intake in pigs and cattle (Adrion et al., 2018; Borchers et al., 2016; Grinter et al., 2019; Kawagoe et al., 2023; Yang et al., 2020; Zambelis et al., 2019). Optical flow analysis of videos of sows can identify feeding behaviour with 95% accuracy (Yang et al., 2020) while UHF RFID can record feeding trough visits in growing pigs with 98% accuracy (Adrion et al., 2018). Studies validating accelerometers on legs, in ear tags and in collars to detect dairy cow feeding behaviour have reported mixed results. Correlation coefficients between visual observations and technological records of cow feeding behaviour ranges from r=27 to r=93 across different studies (Borchers et al., 2016; Grinter et al., 2019; Zambelis et al., 2019). Facial recognition paired with algorithms recorded 83% of observed feeding bouts in dairy cows (Kawagoe et al., 2023). Even in outdoor grazing systems, acoustic technologies have been developed to quantify cattle, goat and sheep feeding behaviour with 94%, 96% and 84% accuracy respectively (Chelotti et al., 2016; Navon et al., 2013). Though the Berckmans (2017) definition of PLF highlights the importance of individual monitoring, there have been advances in PLF approaches to group-level monitoring (Aydın, 2016; Cordeiro et al., 2011), which can be useful in systems where individuals are difficult to identify. For example, an algorithm has been developed to monitor laying hen pecking sounds, using them to provide information on feed intake (Aydın, 2016) and digital image processing can monitor the behaviour of broiler chickens as an indicator of thermal comfort (Cordeiro et al., 2011). If PLF were to become widespread on sheep farms, indicators monitored through technology could eventually be integrated into welfare assurance schemes. Historical and current data could be checked by assessors to identify any behavioural indicators of disease. Further research into the mechanisms behind the behavioural changes reported in this thesis would be necessary for this kind of implementation. For example, the reasons behind the increased standing inactive observed during GI parasitise infection should be examined.

7.2.2 GI Parasitism's effect on mental state

In Chapter 2, QBA results supported the idea that parasitised lambs behaved more fearfully compared to one control group. This is one of the first pieces of welfare science evidence to suggest that GI parasitism has an impact on the mental state of lambs. This could be relevant to the development of future PLF approaches aiming to detect parasitism, as technology could record other fear-related indicators, such as increased distances in a human approach test or an increased latency to feed (Romeyer & Bouissou, 1992). A further application of this new knowledge could be in farmer communication materials around parasitism. Current information focuses on the production and financial cost of parasites and their treatment, but some research indicates that farmers undertake management tasks, such as testing for egg counts before deworming, based on their attitudes towards the importance and value of these tasks (Munoz et al., 2019). Therefore, providing scientific evidence that a disease is negatively impacting sheep's affective state could provide additional motivation for some farmers to treat promptly.

The QBA results in Chapter 2 may also encourage researchers to investigate the third dimension and onwards when conducting Principal Component Analysis (PCA). Regardless of research discipline, choosing the number of dimensions to analyse is a challenge in PCA. It is often decided by conventions (e.g. the dimension explains more than 10% of the variation) or subjectively by each researcher. There are studies that propose various algorithms specifically created to overcome this difficult task (Hubert et al., 2005; Minka, 2000). Although I am not putting forward any specific techniques for selecting the correct number of dimensions, my findings underline the importance of looking past the first two PCA dimensions when working with QBA.

7.2.3 The role of PLF in lameness reduction on sheep farms

This thesis reported a negative association between lameness and lamb grazing behaviour and a positive relationship between ewe lying behaviour and lameness. These findings supported the case for automated lameness detection, for example by accelerometers. This has already been achieved by previous publications, although not with commercially available tools (Abdul Jabbar et al., 2017; Barwick et al., 2018; Kaler et al., 2020). This concept has now been reasonably well tested, and steps to bring lameness detection tools to the market should be undertaken. In 2011, FAWC set a target for 2021 whereby the prevalence of lameness on UK farms should be below 2% (FAWC, 2011). To my knowledge, there has not been a report issued by the Animal Welfare Committee (AWC, FAWC's new name) containing updates on the attainment of these targets or setting new lameness goals for the future. However, sheep farmers, farming organisations, and researchers alike have contributed to reducing lameness through large communication campaigns on best practice, such as AHDB's 5 point plan (AHDB, 2024; Clements & Stoye, 2014). The latest available figure on national prevalence of lameness dates back to 2013, when it was at 4.9% (Winter et al., 2015), but a 2020 survey of 532 sheep farmers places farmer-reported lameness levels at 3.2% (Best et al., 2020). If this figure can be extrapolated and applied to the entire country, then the 2011 target has not been met, and further actions are required. It may be that PLF plays a larger role in lameness detection in the future, as it has been reported that farmers often underestimate lameness prevalence in their flocks (Nalon & Stevenson, 2019). Decisions about whether to treat lame sheep are influenced by human factors such as beliefs and attitudes towards lameness, personality traits and farmer emotional state (Liu et al., 2018; Nalon & Stevenson, 2019; O'Kane et al., 2017) so PLF could potentially offer a more objective identification of lameness cases to be treated. Furthermore, gait score is not perfectly associated with sheep experiencing negative welfare caused by foot lesions (Kaler et al., 2011). In one study, 27% of sheep examined had footrot lesions but were not scored as lame using a gait scoring system (Kaler et al., 2011), meaning that relying solely on gait scoring may miss sheep experiencing poor welfare. Monitoring behavioural changes linked to lameness with technology, such as the ones identified in Chapter 3 of this thesis, may reflect animals' experience better.

In fact, studies have reported that predictive models of disease based on behaviour could be a useful way to apply PLF to disease prevention and treatment, including lameness. These models can analyse large amounts of data and account for different variables easily and accurately to predict future disease events (Neethirajan & Kemp, 2021). They can help farmers manage their animals' health proactively (Neethirajan & Kemp, 2021). For example, a model was trained to detect and predict dairy cow lameness based on leg movement variables using computer vision techniques (Zhao et al., 2018). It predicted lameness cases with 90.25% sensitivity and 94.74% specificity (Zhao et al., 2018). In sheep and goats, machine learning has been used to analyse activity data from accelerometers to predict cases of *Haemonchus contortus* worm infection (Montout et al., 2024). It was found that activity patterns were associated with FAMACHA scores, which are the typically used diagnosis tool for anaemia brought on by *H.contortus* parasitism (Montout et al., 2024). The model predicted cases of infection and allowed for early treatment with a precision of 83% (Montout et al., 2024). If these kinds of predictive models can be developed with high accuracy for the early detection of lameness and other diseases, treatments could be administered before significant harm is done to production levels and animal welfare.

7.2.4 Highlighting ewe welfare

Although much of this thesis reported findings on lamb welfare, Chapter 5 reported that ewe-lamb distance is associated with ewe welfare concerns such as lameness and fleece loss. The distance between a ewe and her young lamb has been used as a measure in the Maternal Behaviour Score (MBS) of ewes, as it is indicative of the strength of the ewe-lamb bond when challenged with the presence of a human (Alexander et al., 1983; Connor et al., 1985). This finding adds a potential welfare assessment tool to the methods for ewe welfare assessment, although visual estimation of ewe-lamb distance in Chapter 3 was not related to any welfare measures. In meat sheep farming, lambs are the source of profit, but ewes are an operation's productive units, meaning they are relied on to produce the farm's source of profit. They can remain on farm for many years and can face regular and possibly additive welfare challenges such as malnutrition, complications around pregnancy and birth or aversive handling experiences (Morris, 2017; Rioja-Lang et al., 2020). Despite their immense value to the longevity of an operation, ewe welfare is sometimes not prioritised due to the low economic value of individuals, the high costs of veterinary treatment and the declining labour force in an already low input system (Kilgour et al., 2008). During the interviews reported on in Chapter 6, when asked about their hopes for the future of PLF, farmers expressed interest in the development of a technology that could measure ewe-lamb distance, as they considered this important information to record in their sheep. There are some commercially available PLF tools for sheep that records this measure, including one discussed by farmers in Chapter 6's interviews, but its use is not widely spread due to the company's history, which caused many farmers to lose trust in them. Though ewes can develop immunity to GI parasitism for example, they accumulate welfare challenges throughout their lives. They should continue to be included in welfare assessments and future studies.

7.3 Limitations to study

7.3.1 Experimental Design

Some animal experiments in this thesis suffered from low statistical power due to small sample sizes. To ensure acceptable statistical power in the indoor study in Chapter 2 for example, nearly double the number of animals would have been necessary. For this study, I was able to reduce the number of animals needed in trials by observing animals already obtained for another PhD student's disease trial, allowing me to assess behavioural changes of animals experiencing known GI infections and to adhere to the reduction principle of the Three Rs (Russell, 2005). However, this meant I did not have control over sample sizes. Similarly, for the trial in Chapter 5, the same ewes that had been part of various other PLF experiments at SRUC's Hill and Mountain Research Centre were used. The study in Chapter 3 benefitted from the fact that data from two years were combined, leading to n=56 and an acceptable power of 84%. Despite the acceptable sample size in the Chapter 3 study, the data still had few recordings of welfare challenges leading to limited variation.

The lack of variation in the welfare data reported in Chapter 3 was likely caused by the experiment relying on natural infection. There was moderate variation when looking at a single welfare issue, for example in the number of lambs scoring 0, 1,2, and 3 on the lameness scale. However, it was variation when looking at multiple welfare issues at once that was limited. For example, if we examine the cross-section between strongyle infection and lameness: out of 981 observations of lambs with no strongyle infection in Chapter 3, only 9 (or 0.9%) had a lameness score of 1. This made studying interactions between welfare issues nearly impossible. Relying on natural infection allows experiments to illustrate commercial conditions and avoid unnecessary challenges to the animals' health and welfare that can occur with induced infections, which aligns with the refinement tenet of the Three Rs, as it likely decreases the incidences of negative experiences for the animals being used. However, there is a risk that the studied challenge will not naturally occur to a statistically significant level, which is what often happened in my trials. I was also unable to control when infections began and ended, or how severe they were. It can result in more variable infection results, although it was not the case in this thesis, and add to the requirement of large numbers of animals to reach statistical significance (Colby et al., 2017).

My experience in qualitative social science is limited, meaning I relied heavily on guidance from a professional social scientist in planning and writing Chapter 6 of this thesis. Despite this, I believe the analysis was rigorous and the findings are important. The language barrier during the interviews in Norway was generally not a problem, although it is possible that certain concepts, like the human-animal relationship, were not communicated perfectly. It is natural that when expressing themselves in their second or third language, participants may have struggled to accurately describe intangible concepts like feelings or cultural phenomena.

7.3.2 PLF Technology

Since validation is a key process for PLF development, it was important to validate all the technology used in this thesis before applying it in welfare studies. However, this meant that rather than testing AX3 accelerometers' ability to detect welfare issues as originally planned, this thesis focused on validating them. As described in Chapter 4, this proved to be a challenge, albeit a necessary one. Working with "home-made" technology, meaning without commercial readers, collars etc., led to a certain level of technical trouble and data loss. For example, some accelerometers and BLE beacons were lost and never recovered. Others simply stopped recording data for unclear reasons. This reduced the contribution of some animals to the datasets. However, small datasets were not an issue with the PLF tools. The entire AX3 dataset was so large that I was never able to open it in its entirety for review or analysis. I was limited to working on it in pieces as my computer did not have the memory available to open such a large file, even when additional RAM (Random Access Memory) was added. It is likely that only a supercomputer, such as the type used for large genomic datasets, would be able to open and analyse files of this size. Access to this type of supercomputer would have been difficult and expensive to obtain. If advanced computational methods are required for the AX3 data to be interpreted, their direct applications on farm are likely limited. It is more likely that algorithms developed with the help of a supercomputer and other processes would be combined with the AX3 to create an entirely new tool that could be useful on farm. Even with only small data sections available, I lacked the advanced machine-learning coding skills to process the data efficiently. I gained many statistical skills that helped me come to grips with the accelerometer datasets, but any commercial applications for detection of behavioural change would likely require complex machine learning algorithms that were beyond the scope of this project.

7.4 What does the future hold for PLF Development?

7.4.1 PLF's relationship with animal welfare

One of the priorities of this thesis was to monitor any direct effects of collars on sheep behaviour and welfare while testing them. This step is often missed when PLF tools are developed from an engineering perspective rather than an animal welfare one. There is evidence of humans altering wild animal behaviour significantly by making them wear identification bands or radio collars. Metal rings to identify birds have been reported to cause injuries to legs through abrasion and accumulation of ice or faeces (Calvo & Furness, 1992). Plastic leg bands of different colours affected the mate preferences of zebra finches (Burley et al., 1982) and radio collars reduced the average body condition score of badgers (Tuyttens et al., 2002). Though livestock usually benefit from closer supervision than wild animals wearing technology, the same risks are present and must be monitored. In indoor environments like pig houses, over 80% of PLF tools being researched are "non-invasive", such as cameras or microphones (Larsen et al., 2021). Such non-invasive, or non-wearable, solutions are more difficult to develop for outdoor, extensive environments but warrant consideration. This is especially true given the findings of Chapter 4 on the effects of collars on ewe behaviour and lamb mental state, and the fact that collars caused lesions in some ewes in Chapter 5. Future PLF studies must continue to collect data and report on the impact of making animals wear objects designed by humans.

Despite these risks, PLF is still often heralded as the next big solution to all of livestock farming's challenges, including prioritising animal welfare. This seems to echo a larger societal pattern where technology is the solution to all global issues: climate change, food insecurity, etc. However, there are well-documented limits to the solutions technology can provide across all sectors. For example, economist William Stanley Jevons theorised that increased efficiency can lead to increased resource use (Jones, 2023; York & McGee, 2016). He came to this conclusion when he noticed that while technological advances during the industrial revolution led to more efficient use of coal, an increase in coal consumption followed (Jones, 2023; York & McGee, 2016). This relationship is referred to as Jevons' paradox, and could be applied to PLF. There is a risk that increasing the efficiency of welfare monitoring on farm (e.g. through automated weighing) could lead to an intensification of livestock farming. This phenomenon was documented in arable farming where intensification through precision technology led to more land use changes and deforestation (Ceddia et al., 2013; Hertel, 2012). It is important to note that the Jevons' association - the correlation between increased efficiency and increased consumption - is the result of multiple interactions (Hertel, 2012). Technology is not the only problem, nor is it the only solution (Jones, 2023).

Human-animal interactions are central to livestock farming and therefore livestock welfare. These interactions currently encompass a variety of management procedures, feeding, and treatments as well as less procedural contacts such as a human petting an animal that has approached them. They are likely to elicit a range of positive and negative affective states in animals. There is a risk that using technology to monitor the animals' welfare could reduce these points of contact to only include ones causing negative affective states such as restraining an animal to give oral treatments for a condition flagged by the technology rather than through in-person observation (Buller et al., 2020; Hostiou et al., 2017; Wathes et al., 2008). The physical distance between humans and animals is likely to increase with additional automation and animals may become more fearful of farmers, negatively impacting their welfare (Boivin, 2012; Hostiou et al., 2017a). There is evidence from farmer interviews that some PLF users felt that the human-animal relationship had deteriorated (Hostiou et al., 2017).

On the other hand, a positive impact of PLF on the human-animal relationship may be that producers can use the time saved on tasks to grow closer to their animals. This was mentioned by participants in the Chapter 6 interviews: automated feed dispensers in barns allowed farmers to observe their sheep closely. Similar results have been reported in studies of dairy farms where automatic milking robots have been installed (Cornou, 2009; Hostiou et al., 2017, 2017a). The new data provided by PLF technology can also help farmers gain individual understanding of their animals, potentially increasing individual interventions such as early treatment of disease, and can increase the satisfaction they gain from their work with animals (Cornou, 2009; Fleuret & Marlet, 2014; Hostiou et al., 2017a).

The presence of PLF systems on farms may interest and motivate future generations of farmers, who perhaps previously thought of farming as an unattractive, "old-fashioned" career. The increase of technology on farm has changed the image of the profession to make it seem more modern (Hostiou et al., 2017). Chapter 6 of this thesis, along with other published studies of farming families, reported that producers invest in technology in attempts to secure their children's future on the farm by creating new revenue streams, but also by integrating the types of modern tools younger people are attracted to (Inwood et al., 2013). In a sector where nearly 40% of farmers are over 65 years old, any factors that attract newcomers or keep younger generations in the business can be seen as positive (DEFRA, 2022; Tuyttens et al., 2022).

Additionally, it is undeniable that technology can do certain things better than humans. For example, repetitive activities such as counting or scoring hundreds of animals can fatigue the human brain whereas an algorithm does not get tired. Retaining high volumes of information is challenging for our memory, but a computer can save huge amounts of data with one click. By using PLF, producers are able to gather relevant information about their animals in a continuous way, thereby allowing a more in-depth knowledge of their needs (Norton et al., 2019). These advantages make it clear that PLF technology is a tool to support humans in their work on animal welfare, rather than the solution to all animal welfare challenges.

7.4.2 The Importance of Validation

As mentioned in Chapter 4, the percentage of PLF tools that are appropriately validated is abysmally low. This can occur when engineering outcomes are prioritised over biological ones during development. Some processes are tested in a limited range of circumstances, for example only inside a barn, but are rolled out advertising the possibility for use in any farming environment (Larsen et al., 2021). Testing in multiple environments is essential if the tool is to be marketed for use other than in one specific system (Stygar et al., 2021) and validation on a reliable number of animals is required. Testing in rough conditions (where the tool is prodded by animals and exposed to weather) should also be conducted, as I identified in Chapter 6 that farmers require their technology to be physically robust. Many factors contribute to the lack of validation studies published, including the possible lack of interest in validation outside of research, the high cost and labour demands of collecting validation data, and a reluctance to publish negative results (Stygar et al., 2021). These issues are amplified when commercial products take the "black box" approach, where the PLF tool produces an index or number on an unknown scale through an unknown formula with unnamed inputs. This process demands that users trust the output without fully understanding how it was obtained. In an ideal world, PLF developers would prioritise transparency to address some of the ethical issues discussed in the literature (Elliott & Werkheiser, 2023). However, developers face the challenge of having limited information available to estimate return on investment for their buyers (Ramirez et al., 2019) and in the realistic context of commercial sensitivity, it is logical that companies would seek to protect their intellectual property and prioritise economic feasibility (Elliott & Werkheiser, 2023; Ramirez et al., 2019).

7.4.3 Including Farmers in the Development of PLF

Another factor that is rarely mentioned in papers describing the development of PLF is farmer priorities. It is often assumed that farmers accord the most importance to economic costs and savings when purchasing technology. While it is undeniable that finances play a large role in their decisions, Chapter 6 of this thesis has added to the evidence that strictly economic models cannot account for the complexity of farmers' motivations and behaviours (Flett et al., 2004). Many theoretical frameworks have been applied to farmer adoption of technology, including the Technology Readiness Index (Parasuraman, 2000) and the Theory of Planned Behaviour (Ajzen, 1991). These illustrate just how multifaceted farmers' motivations and behaviours are. Appropriate market research and stakeholder inclusion, like the work TechCare carried out with the National Stakeholder Workshops, are key to developing PLF approaches that will address farmers' priorities (Czizster et al., 2022; Sossidou et al., 2021). Wearable sensor companies that consult consumers to identify real-world needs have a competitive edge (Peake et al., 2018) and targeting customer needs is a requirement of successful innovation (Baxter, 2017). Beyond identifying stakeholder needs, there is a lack of publications describing the steps of PLF implementation on farms (Larsen et al., 2021). These types of articles could not only guide research priorities but provide a useful resource for curious farmers who would see themselves reflected in the PLF literature. However, there is a balance to be struck between including farmers in PLF development and rolling out technology at their expense (Wathes et al., 2008), as identified in Chapter 6 where participants complained about the experimental nature of some of the technology they purchased. One approach to PLF development that has been suggested in the literature includes a selective process of identifying useful applications, a team including researchers and manufacturers, and a bioethical analysis (Wathes et al., 2008). The authors underline that PLF is too important a tool for sustainable production to risk its rejection due to an inappropriate roll-out (Wathes et al., 2008).

7.4.4. The Ethics of PLF

The enthusiasm for PLF methods can sometimes make it seem like a panacea of solutions to all of livestock farming's challenges. While it has many applications in farming and research, PLF should not be seen as a replacement for more traditional techniques for collecting data and raising animals, but rather as a tool to support farmers and researchers. A paper on wild animal ecology emphasises that the true cost of data collection must be evaluated before applying new research methods (Hughey et al., 2018). This is good advice when planning to collect data using technology from livestock. Researchers should consider the technology's impacts on the animal's behaviour, welfare, and social interactions, for example. Many hours of preparing, troubleshooting, installing and uninstalling tools should be accounted for. Larger, higher-resolution datasets do not automatically lead to a better understanding of animal's worlds (Hebblewhite & Haydon, 2010). This point is crucial and is a reminder that more PLF is not the answer to everything.

Additionally, a very basic question must be answered: do farmers want PLF? The concern of technology push has been previously raised and researchers have questioned whether adequate market research is being conducted (Wathes et al., 2008). It seems technological change across most industries is mainly driven by technology push rather than user demand (Hötte, 2022). The world is increasingly connected across all types of devices through wireless networks (Porter & Heppelman, 2014). The growth of PLF is an offshoot of this broader development, and as a result, many agri-technology companies are being created that push an increasing number of products onto the market (Lesser, 2014; Wolfert et al., 2017). However, shocks in upstream industries (such as the increased global demand for meat, for example) can drive technological innovation in downstream industries (e.g. PLF for increased efficiency in livestock production) (Hötte, 2022; Wolfert et al., 2017). Global food security is often cited as the main driver of PLF innovation (Wolfert et al., 2017) and PLF applications can provide detailed, farm-specific data to help farmers rise to this challenge (Sonka, 2015; Wolfert et al., 2017). Whether farmers want it or not, advancements in technology may mean that the market for PLF has been created and it is here to stay.

The risk that PLF approaches will create barriers between farmers and their livestock by reducing the opportunities for contact has been highlighted by many publications and has previously been discussed in this thesis in 7.4.1 (Buller et al., 2020; Hostiou et al., 2017; Wathes et al., 2008). Interestingly, in Chapter 6 of this thesis, farmers using PLF were aware of this risk but did not feel it applied to them. They prioritised their relationship with their animals despite the PLF technology, or sometimes thanks to it. However, in many cases, it seemed that their ways of building relationships with their sheep was one-sided. It involved observing the sheep during feeding or making more informed decisions about flock health. Farmers often reported that an increased sense of control went together with a good human-animal relationship, which underlines the fact that Chapter 6's study only collected data on this relationship from the farmer side.

7.5 Opportunities for future study

7.5.1 Behaviour and welfare research opportunities

Disease trials where sheep are put through a controlled infection that challenges their welfare would avoid the low variation issues in this thesis brought on by natural infection. For this to occur, partnering with research groups studying disease processes or vaccines can create a situation where animal models are already being used and sampled, and welfare scientists can observe them or test PLF devices while minimizing additional welfare risks. This approach would align with The Three Rs principle of animal research by reducing the number of animals necessary for research questions to be answered (Russell, 2005).

This thesis aimed to identify when behavioural change occurs in sheep experiencing welfare challenges. Time was accounted for in the models of behaviour through the inclusion of scan number and day of experiment as variables, and most temporal patterns fit expectations, e.g. lambs increased their grazing over time. However, a statistical approach centred more explicitly around time would be interesting to reveal any temporal patterns of behavioural change that the GLMMs did not identify. For example, time-series analysis is good at detecting anomalies in data collected over time and can be used in forecasting. It could be used to describe the main feature of normal behaviour, and identify patterns, anomalies or outliers. In a similar vein, the k-means clustering analysis of AX3 validation data could be taken further by training the algorithm on a larger dataset, for example the entire AX3 dataset collected across 2021 and 2022 for this thesis, to refine the cluster selection and potentially improve the validation work.

The result from Chapters 2 and 3 whereby parasitised lambs were more likely to be observed standing inactive than healthy lambs creates an opportunity for accelerometer-based studies to help identify parasitism in lambs. It would be interesting to quantify the reported difference in standing behaviour further, for example through focal observations to quantify the amount of time spent standing still that would trigger an alert from a PLF tool. Alternatively, focal observations of parasitised lambs could be used to further describe this standing behaviour to include details about posture, facial expression and other visual cues that could complete the picture of standing inactive in parasitised lambs. These quantitative measures could be complimented by performing QBA on parasitised lambs and linking the duration of their standing behaviour to arousal levels or valence of their mental states. I attempted to run models investigating the relationship between behaviours in Chapters 2 and 3, including standing inactive, and lamb loadings on QBA dimensions but model fit was poor or the low level of variation in the datasets stopped them from running.

7.5.2 PLF Research opportunities

While studies applying PLF to negative welfare challenges have proliferated in the last decade, positive welfare is less present in the PLF literature (Larsen et al., 2021; Spigarelli et al., 2020). This may be because expressions of positive welfare often entail more complex behavioural patterns. However, if our aim is to obtain a holistic view of welfare through PLF, we must consider not just monitoring and preventing negative experiences but also capturing and creating opportunities for positive ones (Buller et al., 2020; Llonch et al., 2015). For example, measuring heart rate to monitor the valence (positive, neutral or negative) of human-animal interactions could provide insight into positive welfare experiences (Llonch et al., 2015). Other publications have suggested that PLF could create positive experiences through technologies that allow animals to make choices, like automatic feeders or robotic milking, since the ability to make choices has been argued to improve quality of life (van Erp-van der & Rutter, 2020). Such research into positive welfare through a PLF lens is lacking but could contribute to our understanding of the links between PLF and welfare.

In intensive systems, non-invasive PLF such as cameras or microphones are much more common than wearable sensors (Larsen et al., 2021). Though it is much more difficult to imagine these types of tools in extensive environments, they should not be entirely written off. New types of cameras are being developed that have wider lens angles, more capacity for motion or automatic uploads to the cloud, and alternative sources of power such as solar panels. Ultrahigh frequency (UHF) gates have the potential to detect the absence or presence of individuals around a resource such as a salt lick or at gate crossings. Research on UHF gates is ongoing, notably by the TechCare team. It is important to note that PLF research requires collaboration across fields (Norton et al., 2019); ethologists, veterinarians, computer scientists, social scientists and statisticians collaborated towards the work presented in this thesis, for example. This is promising as interdisciplinarity continues to be encouraged by funders and institutions and can lead to research results that are readily applicable in many disciplines.

7.6 Concluding remarks

This thesis has identified potential behavioural welfare indicators for ewes and lambs in extensive production systems, namely that lambs standing inactive is associated with parasitism and ewe lameness is associated with ewes lying. Lambs were also recorded as standing more often when parasitised in an indoor environment. These behaviours and other indicators could be monitored by PLF tools such as accelerometers and BLE beacons. However, validation must be completed, as AX3 were only partly validated for the purpose of identifying different sheep behaviours. A risk that collars containing technology could affect ewe rumination was identified and must not be ignored. Bluetooth beacons were able to monitor ewe-lamb distance in a lambing field, which was associated with ewe welfare scores. Finally, farmers expressed their interest in PLF due to the time and energy savings it offers, because it made them feel more in control of their flock and because it satisfied their curiosity. This thesis' findings are relevant for future welfare assessment research and PLF development.

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Appendices

Appendix A. The impact of gastrointestinal parasitism on the behaviour and welfare of weaned housed lambs

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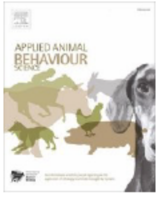

The impact of gastrointestinal parasitism on the behaviour and welfare of weaned housed lambs

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ABSTRACT

Gastrointestinal (GI) parasitism is a health and production concern in sheep, yet its impact on animal welfare remains unclear. The impact of subclinical infections is especially ambiguous as GI parasitism often remains undiagnosed until clinical signs such as diarrhoea are evident. This study applied quantitative and qualitative methods to examine the effects of subclinical *Teladorsagia circumcincta* infection on the behaviour and welfare of 96 Suffolk-cross lambs (24 pens of 4 lambs) weaned at 10 weeks old. The hypothesis that parasitism causes negative affective states was tested. Lambs were divided into three groups at the pen level: ad-lib fed control (AC), restricted-fed control (RC), and ad-lib fed parasitised (AP). Parasitised lambs (AP) were dosed three times weekly with 7000 third stage *T. circumcincta* larvae (L_3) from 16 weeks of age. Lambs in the RC group were pair fed to match AP feed intake to separate the effects of infection-induced anorexia from the potential direct impacts of infection. From 7 days pre-infection to 23 days post-infection, scan and behaviour samples were taken from video recordings to quantitatively monitor behaviour, and animal-based measures such as faecal soiling score (FSS) were recorded as welfare indicators. Lying, standing, eating, play and social behaviour were monitored. Qualitative behaviour assessment (QBA) was conducted weekly using the AWIN (2015) protocol to gain insight into the lambs' affective states over the onset of infection. Parasitised lambs were more likely to stand inactive than AC lambs as the infection progressed (P=0.006). They were also less likely to display eating behaviour in the third daily scan sample than RC lambs (P<0.001). Principal Component Analysis of the QBA data revealed that the first dimension (PC1) described arousal levels, the second (PC2) described the valence of the animals' affective states, and the third (PC3) described fearfulness and aggression levels. Parasitised lambs (est=10.64, SE=0.33) scored higher than RC lambs (est=9.42, SE=0.33) on PC3, the fearfulness dimension (P=0.030). There were no differences between fearfulness scores of AC and AP lambs or RC lambs and treatment group had no significant impact on the distribution of scores on PC1 or PC2. These findings demonstrate that subclinical GI parasitism negatively impacts lamb welfare not only in the health domain but in the behaviour and mental domains as well. This has implications for welfare assessments and early disease detection in lambs. Future research could explore remote monitoring of the indicators of parasitism identified in this study.

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1. Introduction

Gastrointestinal (GI) parasitism is a health and production concern in sheep, costing the United Kingdom's sheep industry €47 million annually (Charlier et al., 2020; Coop et al., 1985). This cost may rise in the future as the UK's grazing season is predicted to lengthen due to climate change, increasing the time during which sheep are exposed to GI parasites (Phelan et al., 2016). However, this condition's effects on behaviour can be difficult to monitor on farm and evidence of its impact on animal welfare is sparse. If lambs adjust their behaviour in early infection it may be possible to use these adjustments as early indicators of parasitism. Early indicators are by definition present and identifiable before the symptoms of clinical disease are visible. Subclinical infection is defined as the presence of parasites in the gastrointestinal tract without the presence of clinical signs such as diarrhoea (Gunn and Irvine, 2003). Without prompt treatment, subclinical GI parasitism leads to morbidity rather than mortality (Kenyon and Jackson, 2012), extending the duration of its welfare impacts. Behavioural effects include anorexia, and changes in diet selection, grazing and social behaviour (Hutchings et al., 1999, 2000b; Morris et al., 2022). Ewe lambs infected with *Teladorsagia circumcincta* spend less time grazing each day and have a lower feed intake than non-parasitised sheep due to their shorter grazing bouts (Hutchings et al., 2000a). Parasitised lambs have lower activity levels and fewer social interactions (Morris et al., 2022). Lying behaviour increased in parasitised lambs in one indoor study (Hempstead et al., 2023), but decreased in a study on pasture (Hogberg et al., 2021¨). Animal-based indicators are the most appropriate tools to provide insight into the welfare state of animals (EFSA, 2012; Smulders and Algers, 2009). Since behavioural symptoms are often visible before clinical signs, studies of sheep behaviour can provide insight into the animals' experiences of welfare challenges (Gougoulis et al., 2010). Qualitative methods like Qualitative Behaviour Assessment (QBA) can complement these approaches by directly assessing animals' affective states.

The experience of subclinically infected lambs remains unclear despite that when asked to rank sheep welfare concerns, UK stakeholders consistently name parasitism as a top issue (Dwyer et al., 2021; Rioja-Lang et al., 2020). There are few studies using an animal welfare science approach to assess the impacts of GI parasitism. The term "affective state" is used here to describe the subjective experience of an animal caused by bodily events and external stimuli (Panksepp, 2005). This study grounds itself in the Five Domains framework, which uses affective states as a measure of the experiment's overall impact on welfare (Mellor, 2016). The five domains are nutrition, environment, health, behaviour and mental state, and the interaction between them provides a systematic assessment of animal welfare (Mellor et al., 2020). By collecting data on the health, behaviour and mental domains of parasitised lambs, we aim to gather information on lambs' affective states during the early stages of infection. Understanding the welfare costs of GI infection by identifying which domains are impacted could help centre it as a welfare issue, as well as a production issue. There is some evidence of altered affective state in ewes infected with Strongylids: they were scored as more "depressed/suspicious" and "unsettled/apprehensive" than non-parasitised ewes using QBA (Grant et al., 2020). Reliable tools to measure the effect of parasitism on welfare are needed to manage it effectively and address welfare concerns. Infection is ubiquitous, and treatment relies on regular anthelmintic treatments (Morgan and van Dijk, 2012). As resistance to anthelmintic drugs increases, the risk of clinical disease rises (Barger, 1999). By treating only infected sheep, in refugia parasite populations parasites are preserved and the anthelmintics' efficacy is prolonged (Kenyon et al., 2009). Targeted selective treatment is a method of identifying individual infected animals based on production factors such as live weight gain (Kenyon et al., 2009). More tools for early identification of infected sheep are needed to avoid blanket treatments of entire flocks.

The aims of this study were to identify early behavioural indicators of *T.circumcincta* infection through scan and focal sampling, and to explore its impact on lamb welfare through QBA, behavioural change and welfare indicators like faecal soiling score (FSS) and gut fill score (Phythian et al., 2013, 2019). We hypothesised that infected lambs would reduce their activity levels, feeding and social behaviour compared to non-infected lambs. They would have higher FSS and lower gut fill scores. We hypothesised that QBA would capture infected lambs' negative affective states through higher scores on terms like `listless` and `apathetic.`

2. Methods

2.1. Ethical approval

Ethical approval for this study was granted by SRUC's Animal Experiment Committee, as a subset of a larger experimental trial (AE Number: SHE AE 03–2021). Humane end points for parasite infection were set in the ethical approval documentation. These outlined that any lamb showing profuse diarrhoea for more than 24 hours will be given veterinary treatment, including anthelmintic drugs. However, the parasite dose administered was not expected to result in severe clinical disease and no animals reached this endpoint throughout the trial. All work is reported to be fully compliant with the ARRIVE2.0 guidance.

2.2. Animals

Ninety-six Suffolk cross male (48) and female (48) lambs were studied in this experiment. Eighty-four were Suffolk X Texel and the remaining twelve were Suffolk X Blueface Leicester lambs balanced across the three treatment groups described below. All but five of the lambs were twins so the singletons were balanced across treatment groups. They were born within 10 days of each other on the experimental farm and remained with their dams until weaning at 10 weeks of age. All lambs had tails docked and males were castrated. They were housed indoors until the experiment began when they were 4 months of age to ensure they were naïve to GI parasites. Prior to the start of the study, lambs were fed commercial pelleted feed (Tarff Valley Ltd., Castle Douglas, UK). During the study, they were housed in a naturally ventilated shed where 24 pens were made of metal railing in blocks of four, each block being separated by a walkway. Lambs were kept in groups of four according to their treatment in the pens with a space allowance of 1.96 m^2 per lamb. Each pen contained at least four feeders and one drinker, with saw dust bedding. Pens were bedded with fine wood shaving and completely cleaned out every 8 days, with daily fresh bedding added as necessary.

2.3. Experimental design

2.3.1. Treatment groups

There were three experimental treatments with 8 replicates, each consisting of a pen of 4 lambs balanced for live weight. Computer programming (RStudio) was used to allocate lambs to each treatment group. Lambs were initially ranked according to starting trial weight, then grouped together to minimise weight difference between each replicate of 4 lambs per treatment. The treatment groups were ad- libitum fed control (AC), restricted-fed control (RC), and ad-libitum fed parasitised (AP). The latter were orally trickle dosed three times per week (with an interval of 2 or 3 days) with approximately 7000 *T. circumcincta* L_3, a dose known to lead to subclinical infection (Coop et al., 1982; Fox et al., 2018). The AC and RC groups were sham infected with 4 mL of water, following the same protocol as the AP group. The first doses of larvae and sham doses were administered to lambs, pen by pen, on a rolling basis over 6 days. Infection was monitored through faecal egg counts every 10 days from the various days of first infection for each pen using the modified flotation method with a sensitivity of one egg per gram (epg) of faeces (Christie and Jackson, 1982). Feed intake for the ad-lib fed parasitised lambs and ad-lib fed control lambs was measured daily. Feed intake per pen was recorded based on systematic weighing of feed given and leftover feed. The lambs' diet was made up of grass pellets (For Farmers UK Ltd., Bury St Edmunds, UK) consisting of 939 g/kg of dry matter and 122 g/kg DM of crude protein. They were fed once a day between 9 and 10 am. The RC group was restricted-fed to match the feed intake of the parasitised group, on a 3-day rolling average basis, as they developed parasite-induced anorexia. This meant that after the onset of infection, RC lambs were given a restricted diet. This was to control for the confounding effect of anorexia and allow for the assessment of the true impact of parasitism on behaviour and welfare. The mean daily feed intake of the parasitised group over the previous 3 days was calculated to smooth-out natural fluctuations in daily feed intake and this calculated amount of feed was given to the RC group. Mean feed intake was recalculated for the RC group on a daily basis since both the growth of the lambs and the degree of parasite-induced anorexia impacted the daily feed intake of the parasitised lambs. Once restrictions were in place, RC pens had 5 feeders to minimise fighting. Before the beginning of the trial, the mean body weight of AC lambs was 29.6 kg, while RC lambs weighed 30 kg and AP lambs weighed 29.9 kg on average.

2.3.2. Parasitology

Lambs on-farm (but outwith the present trial) were inoculated with *T. circumcincta* to maintain a supply of fresh parasite larvae for the trial. Faeces were collected daily throughout the week using collection bags, then incubated in stable conditions for at least 10 days before the hatched L_3 larvae were collected using the Baermann technique (Walker and Wilson, 1960). The quality and quantity of larvae collected was visually assessed using microscopy, then the larvae were stored in water at 5 °C until they were about to be used. Prior to use, the concentration of viable larvae was assessed using microscopy and either concentrated or diluted to ensure that 7000 viable L_3 would be given within a 3–5 mL volume of the suspension. The consistency of the larval concentration was checked prior to dosing the trial lambs. Anthelmintic treatment was given to all lambs in the days immediately prior to them being moved into the trial location for a settling-in period, and infected lamb were treated again at the end of the trial.

2.4. Data collection

2.4.1. Video recordings

Data collection occurred over 4 weeks, from day of infection (DOI) − 7 pre-infection to 23 post-infection. Twelve cameras were placed on posts above 4 pens of each treatment (16 lambs/ treatment) and connected to a computer running GeoVision surveillance software (GeoVision Inc., Taipei, Taiwan). Each camera clearly captured the entirety of one pen. Video was recorded every day for one hour between 13:00 h and 14:00 h for 28 days. This time slot was selected through observing 48 hours of continuous video footage captured one week prior to the beginning of the experiment and selecting the time of day where video quality was highest and disturbances were minimal. Management and experimental procedures were complete by 1 pm, meaning the lambs were mostly undisturbed, and the natural light in the barn led to good image quality. Video data were downloaded onto a hard drive every other day and uploaded to an institutional server at the end of the experiment. The functioning and placement of the cameras were checked every morning and they were adjusted as needed. The four individual lambs in each pen were identified by a livestock marker paint (Ritchey Livestock ID, Brighton, USA) dot on their shoulders, mid-back, or rump and the fourth lamb was identified by the lack of a marking.

Behavioural sampling from the videos was conducted by a trained observer blind to the lambs' treatment groups using The Observer XT 15 (Tracksys Ltd., Nottingham, UK). The observer had seven years of animal behaviour and welfare research experience and data collection protocols were approved by senior researchers. Three scan samples at 30-minute intervals (minutes 0, 30 and 60 of each video recording) and one 30–minute pen-level continuous focal sample was taken from each daily recording to record social behaviour and play, using the ethogram shown in Table 1. Scan samples were carried out at the individual lamb level while focal samples were conducted at the pen-level.

2.4.2. Qualitative behaviour assessment (QBA)

QBA was carried out on each pen weekly between 11:00 h and 13:00 h, a time chosen to avoid disturbances in the barn. The same observer, blind to the lambs' treatment groups, performed QBA every

Table 1

Ethogram of lamb behaviours collected by scan and focal sampling for penned lambs kept in groups of 4 to determine the effects of parasitism on behaviour, where behaviours without an asterisk (*) were only used in scan sampling and behaviour marked with an asterisk (*) were used in scan and focal sampling.

Behaviour	Definition
Feeding	Lamb has head within 10 cm of the feed or water trough, may be seen biting, chewing or obtaining feed.
Drinking	Lamb has head within 10 cm of the water trough, may be seen to be licking, mouthing the trough or obtaining water from trough.

Locomotion	Lamb moves feet, leading to motion in any direction for more than 2 seconds.
Lying	Lamb's body is touching the ground from shoulder to back end, neck and head touching the ground or upright.
Standing	Lamb remains still in a posture where head is raised above the level of the back, up on all four legs.
Pen Exploration	Lamb nudges, noses or chews any object or structure, other than feed, water, bedding or the brush head.
Locomotor play *	Lamb moves rapidly in any direction for more than 2 seconds with no obvious destination to reach, jumping or pivoting for no obvious reason
Social play *	Lamb puts its head down and runs to butt heads with another lamb, or jumps up onto back legs and rests its front half on the back of another lamb
Social behaviour *	Lamb is in any kind of active physical contact with another lamb, including nudging, nuzzling, or nosing. Excludes passively lying close to another lamb and touching it.
Object play *	Lamb's face is within 5 cm of the brush head, or it interacts with the brush head by sniffing, butting, pawing or jumping on it.
Unclear	Lamb's behaviour is concealed by a visual barrier e.g. feeder or another lamb.

week. The observation protocol was reviewed and approved by senior researchers. After entering or changing positions in the barn, the observer allowed sufficient time for the animals to settle before beginning the observations. For example, if vigilance behaviour began when the observer took their place, observations did not begin until vigilance behaviour disappeared. Once the animals were judged to have resumed their ongoing behaviour, each pen was observed for 2 minutes, starting with the farthest pen and ending with the nearest. The protocol and list of terms presented in the EU Animal Welfare Indicators (AWIN) project Protocol for Welfare Assessment in Sheep (AWIN, 2015) was used to score the lambs' demeanour using a visual analog scale for every term on a tablet (Xperia S, Sony Europe Ltd., Weybridge, UK). Ninety-six pen-level assessments were carried out over four weeks, with each of the 24 pens being observed 4 times.

2.4.3. Live weight, faecal sampling and visual scores

All lambs were weighed on day − 7, 2, 12 and 21 of parasite infection. Before being moved to the weighing area, faecal samples (approx. 6 g per animal) were collected in the pen following natural expulsion of faecal matter. If a sufficient faecal sample could not be obtained naturally, a direct faecal sample was collected. Lambs were then moved to a holding pen linked to a weigh crate. While in the holding pen, FSS and gut fill scores were assigned to every lamb based on visual inspection. Faecal soiling was scored on the scale from 0 to 4 developed by AWIN (AWIN, 2015), where:

- 0: No faecal soiling, the wool around the breech area and under the tail is clean
- 1: A small quantity of faecal matter in the wool around the anus
- 2: Some soiling around the anus and dags (matted areas of faecal matter adhering to the wool) in this area only
- 3: Soiling and dags extending beyond the anus to the tail and onto the upper part of the legs
- 4: Wider area of soiling with dags extending down the legs as far as the hocks.

To record gut fill, lambs were scored as 2 for bloated, 1 for full or 0 for emaciated, as previously described (Phythian et al., 2013). Lambs were then individually weighed and returned to their home pens.

2.5. Statistical analysis

For all analyses, data were separated into pre-infection (DOI − 7 to − 1) and post-infection (DOI 0–23). The pre-infection dataset was used to determine the baselines of feed intake, behaviour and mental state, while the post-infection dataset showed the effect of infection on these variables. Unless stated otherwise in the model descriptions, pen number was included as the random effect in the models. Generalised Linear Mixed Models (GLMM) and cumulative linear mixed models (CLMM) were used to analyse feed intake, behaviour, and welfare indicators because of their ability to process repeated measures taken over time from the same individuals and to handle unbalanced designs, as well as the possibility of include random effects. Fixed and random effects were chosen to answer the research questions and account for possible confounding factors. Missing data were included in the data set as blank cells.

Scan and behaviour samples were exported from The Observer XT 15 into Microsoft Excel. All statistical analysis was conducted in R 4.2.2 (R Core Team, 2022) via R Studio (version 3.0). To determine if changes in feed intake took place, a GLMM [glmmTMB package (Brooks et al., 2017)] was utilised using pen as the experimental unit with negative binomial distribution with a quadratic parameterization (nbinom2) link function. Fixed effects included treatment (AC, RC and AP) and day of infection (DOI) as a covariate, as well as the interaction between the two.

Behaviours performed more than 5 % of the time during scan sampling were analysed. To determine the relationships between the binary behaviours (presence/absence (0,1)) performed during scan sampling and the treatment groups, GLMMs [glmmTMB package (Brooks et al., 2017)] were performed with a binomial probability distribution (binomial) where each lamb acted as the experimental unit. Fixed effects included treatment (AC, RC and AP), scan sample (0, 30 or 60 mins) and day of infection (DOI) as a covariate. Interaction terms included 2-way interactions between *DOI * Treatment*, *DOI * scan*, and *scan * Treatment*. Lamb ID nested within pen number was included as a random effect.

Behavioural analysis during focal sampling included comparisons of total durations and frequencies across treatment groups at pen level (4 lambs combined within pen) for each 30-minute focal sample via GLMMs [glmmTMB package (Brooks et al., 2017)]. Social play, locomotory play and object play were combined to form a single play behaviour response variable. The family link function was set to negative binomial distribution with a quadratic parameterization (nbinom2). Fixed effects were DOI and treatment (AC, RC and AP), as well as an interaction (*DOI * Treatment)*. Pen was included as the random effect. Differences in social behaviour and play were compared between the pre-infection and the post-infection period. Negative binomial GLMMs were also used for this analysis where fixed effects included a factor describing the timing of each observation (pre-infection, post-infection) and treatment group, and an interaction term *timing*treatment* was included.

Principal Component Analysis (PCA) (Wold et al., 1987) was used to explore differences in lamb affective state across treatment groups as assessed by QBA. A PCA was run on the scores for the descriptive terms (21 total) across observations and pens using the R package *stats*. A scree plot was produced using the package *factoextra* (Kassambra and Mundt, 2020) and the three dimensions that accounted for the highest levels of variance (more than 10 %) were retained for graphical representation and modelling. The base R function *print* was applied to the resulting PCA to produce a covariance matrix for the 21 terms and the PCA dimensions. This allowed for interpretation of each dimension. The R package *factoextra* (Kassambra and Mundt, 2020) was used to create graphs of the distribution of pens along the dimensions. It was also used to extract the coordinates of each observation along the first three dimensions. This new dataset contained variables called Arousal, Valence and Aggression, which described the placement of each observation along the respective dimensions. For these three variables, GLMMs were used to evaluate whether the lambs' loadings were related to treatment group or day of infection, with Y+10 to account for negative values in the response variable without disrupting variance. The family link function was set to either negative binomial distribution with a quadratic parameterization (nbinom2) or Gaussian distribution, dependent on model fit and overdispersion parameters (Hardin and Hilbe, 2007). Fixed effects included treatment (AC, RC and RP) and DOI as a covariate, as well as the interaction between the two (*DOI * Treatment)*.

A CLMM [ordinal package (Christensen, 2022) and RVAideMemoire (Herv´e, 2023)] with the threshold set to flexible was used to determine the relationships between FSS and treatment. Model fitness was verified by log-likelihood test in the *ordinal* package (Christensen, 2022). Fixed effects included treatment (AC, RC and RP) and DOI as a covariate, as well as an interaction between the two (*DOI * Treatment)*. Lamb ID nested within pen number was included as the random effect.

For all GLMMs, model fitness, normality of residuals and homogeneity of variance was graphically confirmed using the DHARMa package (Hartig, 2022). The ANOVA function in the *car* package (Fox and Weisberg, 2018) was used to determine the significance of explanatory variables based on a $p < 0.05$ threshold and to examine differences between fixed effects and interactions. Pairwise comparisons of estimated marginal means (i.e. adjusted or least-squares means) and associated standard errors were derived with the *emmeans* function of the *emmeans* package (Lenth, 2023) with mode set to "mean.class" to obtain the average probability distributions as probabilities of the visual scores and "response" to obtain estimates of the probability distribution in the response scale for each treatment group, with Tukey adjustment of p-values accounting for multiplicity. *Emmeans* (Lenth, 2023) was also used to examine linear trends between fixed effects and covariates. Graphical representations of results were produced using *ggplot2* (Wickham, 2016) with corrected pairwise comparisons with standard error (SE) and 95 % confidence intervals (CIs) reported.

3. Results

3.1. Pre-infection results

There was a significant effect of treatment group on feed intake between DOI − 7 and − 1 (mean feed intakes: RC=7931±89.2 g, AC=8580 ±96.5 g, AP=8674±97.8 g, $X^2_{(2,238)}$=37.66, P<0.001), when all animals were being fed ad libitum. There were no significant differences in the likelihood of performing lying behaviour (odds ratios: AC=0.478±0.04, RC=0.516±0.04, AP=0.500±0.04, $X^2_{(2,655)}$=0.33, P=0.850), standing behaviour (odds ratios: AC=0.242±0.004, RC=0.208±0.04, AP=0.161 ±0.04, $X^2_{(2,655)}$=1.81, P=0.404), or eating behaviour (odds ratios: AC=0.204±0.03, RC=0.164±0.03, AP=0.208±0.03, $X^2_{(2,655)}$=1.65, P=0.438) across treatment groups. Analysis of focal samples revealed no significant differences in total durations of play (AC=0.5±0.6 s, RC=0.4±0.9 s, AP=3.5±3.6 s, $X^2_{(2,54)}$=2.58, P=0.276) or social behaviour (total durations: AC=42.1±15.7 s, RC=30.6±11.4 s, AP=37.4±15.1 s, $X^2_{(2,56)}$=0.36, P=0.836), nor in number of bouts of play (mean bout counts: AC=0.05±0.05, RC=0.25±0.18, AP=0.27±0.19, $X^2_{(2,56)}$=4.18, P=0.124) or social behaviour (mean bout counts: AC=3.10±0.85, RC=2.81±0.78, AP=3.30±0.95, $X^2_{(2,56)}$=0.13, P=0.932) between treatment groups. QBA loadings along the arousal dimension increased for all treatments across the pre-infection period, although there was a significant difference in the rate of that increase between AP and RC lambs (slopes: AC=1.300±0.36, RC=1.847±0.33, AP=0.316± 0.32, $X^2_{(2,29)}$=11.61, P=0.007). There was a significant effect of treatment on FSS in the pre-infection period (mean scores: AC=1.86±0.15, RC=1.99 ±0.16, AP=1.70±0.16, $X^2_{(2,29)}$= 40.24, P<0.001).

3.2. Post infection results

3.2.1. Faecal Egg Counts (FEC)

The parasitised treatment group (AP) was the only group whose FEC rose above zero for the entire study period, and only from DOI 11. That day, AP lambs began showing low FECs of 1.4±0.6epg (mean±SE). On DOI 12, AP lambs had a mean FEC of 3.2±0.7epg. Ten days later, on DOI 21, all 32 AP lambs were shedding eggs, with a mean FEC of 77.2 ±14.7epg, and AC and RC lambs' FEC remained at 0. A Kruskal-Wallis test of FEC on DOI 21, the first day of the patent period of infection when lambs are expected to start shedding parasite eggs, found a significant difference between APlambs and RC and AC lambs ($X^2_{(2)}$=90, P<0.001).

3.2.2. Feed intake

Feed intake increased over time for all three treatment groups as the lambs grew. Mean feed intake during the infection period for AC lambs was 10213±72.9 g, 9585 g±54.0 g for RC lambs and 10059±70.3 g for AP lambs. There was a significant effect of the interaction between DOI and treatment group on feed intake ($X^2_{(2,491)}$=11.53, P=0.003). The increase in feed intake over time for AC lambs was significantly greater than for AP lambs (slopes: AC=0.006±0.001, RC= 0.003±0.001, AP=0.001±0.001, Z_{ratio}=3.39, P=0.002). There was no significant difference in feed intake over DOI between RC and AC (Z_{ratio}=1.85, P=0.155) or RC and AP lambs (Z_{ratio}= − 1.52, P=0.281).

3.2.3. Scan samples

Across all treatment groups, the most frequently recorded behaviour was lying (61.6 % of observations), and the least frequently observed was object play (0.01 % of observations). For AC lambs, lying was recorded in 60.6 % of observations while standing and eating accounted for 15.2 % and 16.2 % of observations, respectively. Lambs in the RC group were recorded as lying, standing and eating during 60.8 %, 14.6 % and 17.2 % of observations, respectively. In AP lambs, lying, standing and eating made up 63.6 %, 15.5 %

and 15.7 % of observations, respectively. The other behaviours in the ethogram (Table 1) were seen less than 5 % of the time across treatment groups, and therefore were not analysed.

3.2.3.1. Lying behaviour.

Scan number had a significant effect on lying behaviour (probabilities: Scan 1=0.48±0.02, Scan 2=0.68±0.02, Scan 3=0.70±0.02, $X^2_{(2,2307)}$=95.92, P<0.001). Lying was less likely to occur during scan 1 than scan 2 (OR=0.42±0.04, Z_{ratio}= − 8.11, P<0.001) and scan 3 (OR=0.40±0.04, Z_{ratio}= − 8.64, P<0.001) for all treatment groups. There was no significant effect of treatment group on lying behaviour ($X^2_{(2,2307)}$=1.37, P=0.504) and no significant interaction between DOI and treatment group (($X^2_{(2,2307)}$=0.86, P=0.649).

3.2.3.2. Standing behaviour.

When modelling standing behaviour, there was a significant interaction between DOI and treatment group (slopes: AC=0.02±0.02, RC=0.06±0.02, AP=0.10±0.02, $X^2_{(2,2307)}$=9.55, P=0.008). As shown in Fig. 1, AP lambs were more likely to be standing as DOI increased than AC lambs (est= − 0.08±0.03, Z_{ratio}= − 3.06, P=0.006), especially from DOI 14 onwards. The RC lambs' likelihood of standing behaviour did not differ from AC (est= − 0.05±0.03, Z_{ratio}= − 1.73, P=0.193) or AP lambs (est=0.04±0.04, Z_{ratio}=1.26, P=0.416) (Fig. 1). This means that AP lambs may have reduced their activity levels as infection progressed, if standing is considered an inactive behaviour.

There was a significant interaction between treatment group and scan number for standing behaviour ($X^2_{(4,2307)}$=23.47, P<0.001). Lambs in the AC group showed a significant decrease in likelihood of standing behaviour between scans 1 and 3 (probabilities: Scan 1=0.21±0.03, Scan 3=0.10±0.02, OR=2.26±0.57, Z_{ratio}=3.25, P=0.003), while RC's decreased between scans 1 and 2 (probabilities: Scan 1=0.27±0.03, Scan 2=0.08±0.02, OR=4.21±1.13, Z_{ratio}=5.35, P<0.001) and scans 1 and 3 (probabilities: Scan 1=0.27±0.03, Scan 3=0.07±0.02, OR=4.90 ±1.42, Z_{ratio}=5.48, P<0.001). Lambs in the AP group (probabilities: Scan 1=0.16±0.03, Scan 2=0.10±0.02, Scan 3=0.16±0.03) showed no significant difference in standing behaviour likelihood between scan 1 and scan 2 (OR=1.78±0.49, Z_{ratio}=2.10, P=0.089), scan 1 and scan 3 (OR=1.00±0.25, Z_{ratio}=0.01, P=0.999), or scans 2 and 3 (OR=0.57 ±0.16, Z_{ratio}= − 2.08, P=0.09), meaning they were equally likely to be standing across the entire scan sampling period.

3.2.3.3. Eating behaviour.

There was a significant interaction between treatment group and scan number for eating behaviour ($X^2_{(4,2307)}$=18.54, P<0.001). As illustrated in Fig. 2, during scan 1 (probabilities: AC=0.22±0.03, RC=0.20±0.03, AP=0.27±0.03) there were no significant differences between AP and AC lambs (OR=0.78 ±0.17, Z_{ratio}= − 1.10, P=0.512), nor between AP and RC (OR=1.44 ±0.33, Z_{ratio}=1.62, P=0.239) or AC and RC (OR=1.13±0.26, Z_{ratio}=0.53, P=0.855). During scan 2 (probabilities: AC=0.14±0.02, RC=0.12±0.02, AP=0.12±0.02) there were again no significant differences between AP and AC lambs (OR=1.14±0.31, Z_{ratio}= 0.47, P=0.887), nor between AP and RC (OR=1.01±0.28, Z_{ratio}=0.02, P=0.874) or AC and RC (OR=1.14±0.31, Z_{ratio}=0.50, P=0.874) (Fig. 2). However, during scan 3, AP lambs were significantly less likely than RC lambs to be performing eating behaviour (probabilities: AC=0.12±0.02, RC=0.19±0.03, AP=0.07±0.02, OR=0.32±0.10, Z_{ratio}= − 3.74, P<0.001), which is visible in Fig. 2. This result may reflect the expected parasite-induced anorexia in AP lambs.

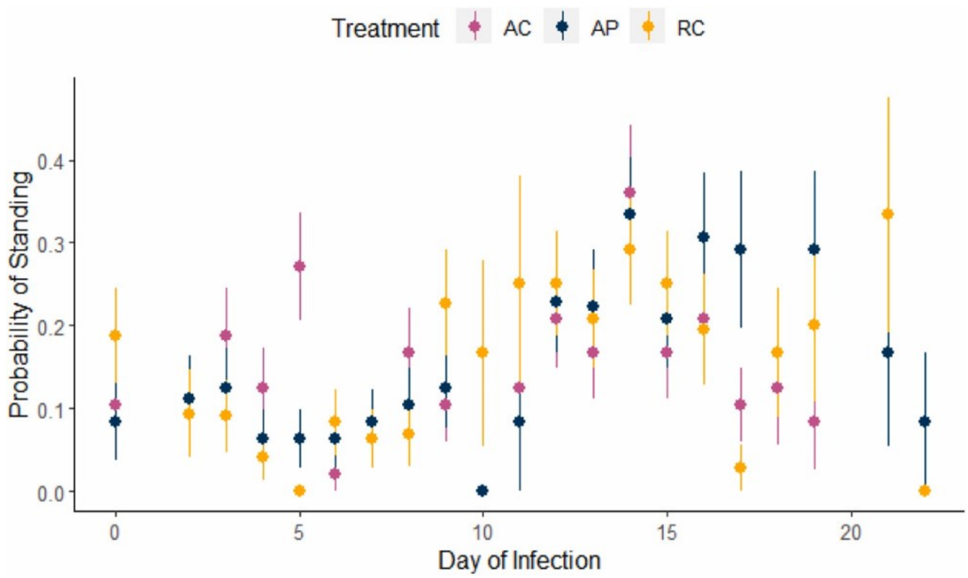

Fig. 1. Mean probability with standard error of lamb standing behaviour by treatment group from day 0 of infection to day 23 of infection, where AC=ad-lib fed control, RC=restricted-fed control and AP=ad-lib fed parasitised.

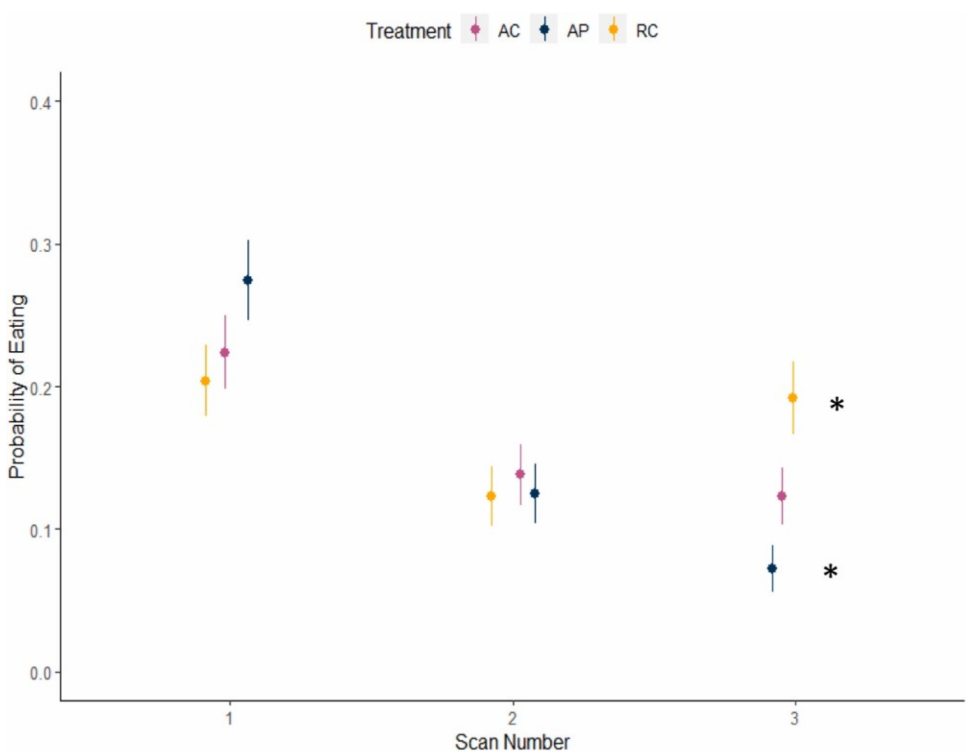

Fig. 2. Mean probability and standard error of lamb eating behaviour across the three daily scan samples by treatment group, where AC=ad-lib fed control, RC=restricted-fed control and AP=ad-lib fed parasitised. Dots with differing star symbols are significantly different from each other.

3.2.4. Focal samples

As a whole, lambs performed social behaviour and play behaviour 295 and 45 times respectively (Table 2). As expected and shown in Table 2, play behaviour occurred less often than social behaviour.

Table 2

Total number of bouts, total duration of bouts, and mean duration of bouts for social behaviour and play at the pen level for the infection period across treatment groups.

	Treatment Group	Play	Social Behaviour
Total number of bouts	AC	21	94
	RC	10	81
	AP	14	120
	Total	**45**	**295**
Total duration of bouts (s)	AC	830.6	856.8
	RC	1279.4	1057.6
	AP	328.3	1244.6
	Total	**2438.3**	**3159.0**
Mean ± SE duration of bouts (s)	AC	2.5 ± 1.3	3.0 ± 0.6
	RC	12.5 ± 6.5	6.8 ± 2.0
	AP	3.2 ± 1.1	4.4 ± 0.8
	Mean	**6.0 ± 1.8**	**4.7 ± 1.8**

There was a significant interaction between DOI and treatment group when modelling total duration of play ($X^2_{(2,24)}$=6.13, P=0.047). As seen in Fig. 3, play bout duration decreased over time for AC and AP lambs but increased for RC lambs (slopes: AC=-0.13±0.07, RC=0.12±0.08, AP= ₋0.06±0.08), especially from DOI 14. The difference in play bout duration trend over time between AC and RC lambs was significant (estimate= ₋0.25±0.10, Z_{ratio}= ₋2.43, P=0.040) though the differences between AC and AP (estimate= ₋0.07±0.10, Z_{ratio}= ₋0.63, P=0.802) and AP and RC were not significant (estimate= ₋0.19±0.11, Z_{ratio}= ₋1.70, P=0.207) (Fig. 3).

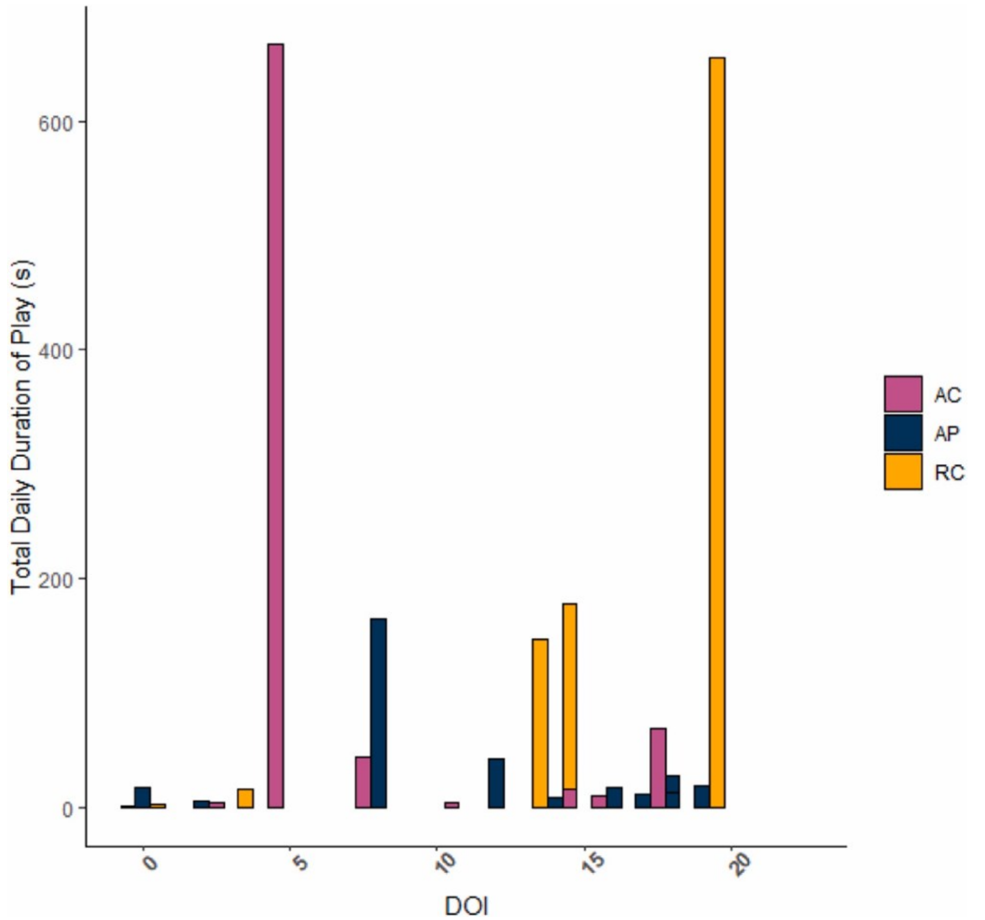

Fig. 3. Total daily duration of play behaviour in seconds every day of infection (DOI) for the three treatment groups, where AC=ad-lib fed control, AP=parasitised and RC=restricted-fed control lambs.

Contrary to what was hypothesised, total duration of social behaviour was not significantly affected by DOI ($X^2_{(1,80)}$=1.39, P=0.239) or treatment group ($X^2_{(2,80)}$=1.04, P=0.0594). The number of bouts of social behaviour was similarly unaffected by DOI ($X^2_{(1,192)}$=0.28, P=0.600) or treatment group ($X^2_{(2,192)}$=0.54, P=0.762). No statistically significant relationships existed between the number of bouts of play performed by each pen and DOI ($X^2_{(1,192)}$=0.003, P=0.956) or treatment group ($X^2_{(2,192)}$=1.39, P=0.500). When comparing before and after infection, there was a significant decrease in the number of social behaviour bouts after infection for all treatment groups (OR=0.45 ±0.11, Z_{ratio}= − 3.41, P<0.001).

3.2.5. QBA

The PCA revealed that principal component 1 (PC1) accounted for 36.7 % of the variance, PC2 accounted for 15.1 % of the variance, and PC3 accounted for 12.8 % of the variance. Cumulatively, PC1, PC2 and PC3 accounted for 64.6 % of the variance in the QBA data.

Table 3 was used to interpret the meaning of the PCA dimensions. PC1 seemed to described arousal levels, with terms such as \`Calm\`, \`Relaxed\`, and \`Subdued\` on one end and \`Active\`, \`Vigorous\` and \`Assertive\` on the other (Table 3). PC2 may have described the valence of the animals′ affective states, running from \`Agitated\`, \`Apathetic\` and \`Physically Uncomfortable\` to \`Content\` and \`Bright\` (Table 3). PC3 suggested it may reflect the spectrum of fear and aggression, running from `Sociable` and `Aggressive` to `Alert`, `Fearful`, and `Tense` (Table 3).

Table 3

Matrix of the 21 QBA terms for pen-level observations. Cells with a single border show the two terms with the highest positive values and cells with a double border show the two lowest negative values.

Term	PC1	PC2	PC3
Alert	-0.1207	-0.2635	-0.2807
Active	-0.2933	-0.0123	0.2037
Relaxed	0.2728	-0.2555	-0.0638
Fearful	-0.1132	0.1860	-0.4681
Content	0.1555	-0.4150	-0.0330
Agitated	-0.1220	0.3182	-0.0968
Sociable	-0.2061	-0.0754	0.2383
Aggressive	-0.1940	0.1129	0.2099
Vigorous	-0.3153	-0.0598	0.1383
Subdued	0.2837	0.2012	0.1028
Physically uncomfortable	0.0742	0.2876	-0.0218
Defensive	-0.1671	0.0629	0.1963
Calm	0.3206	-0.1458	-0.1049
Frustrated	-0.1108	0.2482	-0.0460
Apathetic	0.2560	0.2836	0.1723
Wary	-0.0963	0.0887	-0.4704
Tense	-0.1427	0.2567	-0.3883
Bright	-0.2525	-0.3055	-0.0996
Inquisitive	-0.2703	0.0189	0.0482
Assertive	-0.3010	0.0106	0.1375
Listless	0.2038	0.2766	0.1735

Treatment group had no significant impact on the distribution of pens along PC1 ($X^2_{(2,65)}$=0.09, P=0.956) or PC2 ($X^2_{(2,65)}$=1.13, P=0.569) over the infection period of the experiment, contrary to the hypothesis that parasitism would impact AP lambs' PC2 or valence loadings. However, loadings along PC3, the dimension describing aggression and fear, were different across treatment groups (mean loadings: AC=9.92 ±0.33, RC=10.62±0.33, AP=9.42±0.33, $X^2_{(2,65)}$=6.89, P=0.0.32) (Fig. 4a). Lambs in the AP group had significantly lower PC3 loadings than RC lambs (estimate= ₋1.20±0.46, Z_{ratio}= ₋2.59, P=0.032), meaning they were behaving more fearfully than RC lambs (Fig. 4b). This is highlighted in Fig. 4b by the difference along the Y axis between the placement of the blue (AP) and yellow (RC) ellipses. The AC's PC3 loadings were not significantly different from either RC (estimate= − 0.70±0.47, Z_{ratio}= ₋1.50, P=0.297) or AP (estimate=0.50±0.46, Z_{ratio}=1.09, P=525) (Fig. 4b).

3.2.6. Visual scores

3.2.6.1. Gut fill. All lambs scored a gut fill of 1 (normal fill) at every sampling day throughout the study, so no analysis of the score's relationship with parasitism could be conducted and the gut fill hypothesis could not be tested.

3.2.6.2. Faecal soiling scores (FSS). For all treatment groups during the infection period, FSS 1 was most often recorded, and FSS 4 was only recorded 5 times. The AC group had a median FSS of 3 (IQR=2), RC lambs' median FSS was 2 (IQR=1) and AP lambs' median FSS was 2 (IQR=1). FSS increased over time across all treatment groups ($X^2_{(3,90)}$=36.34, P<0.001) but there was no significant effect of treatment group on FSS ($X^2_{(2,90)}$=3.84, P=0.147), leading us to reject the hypothesis that parasitised lambs would have higher scores.

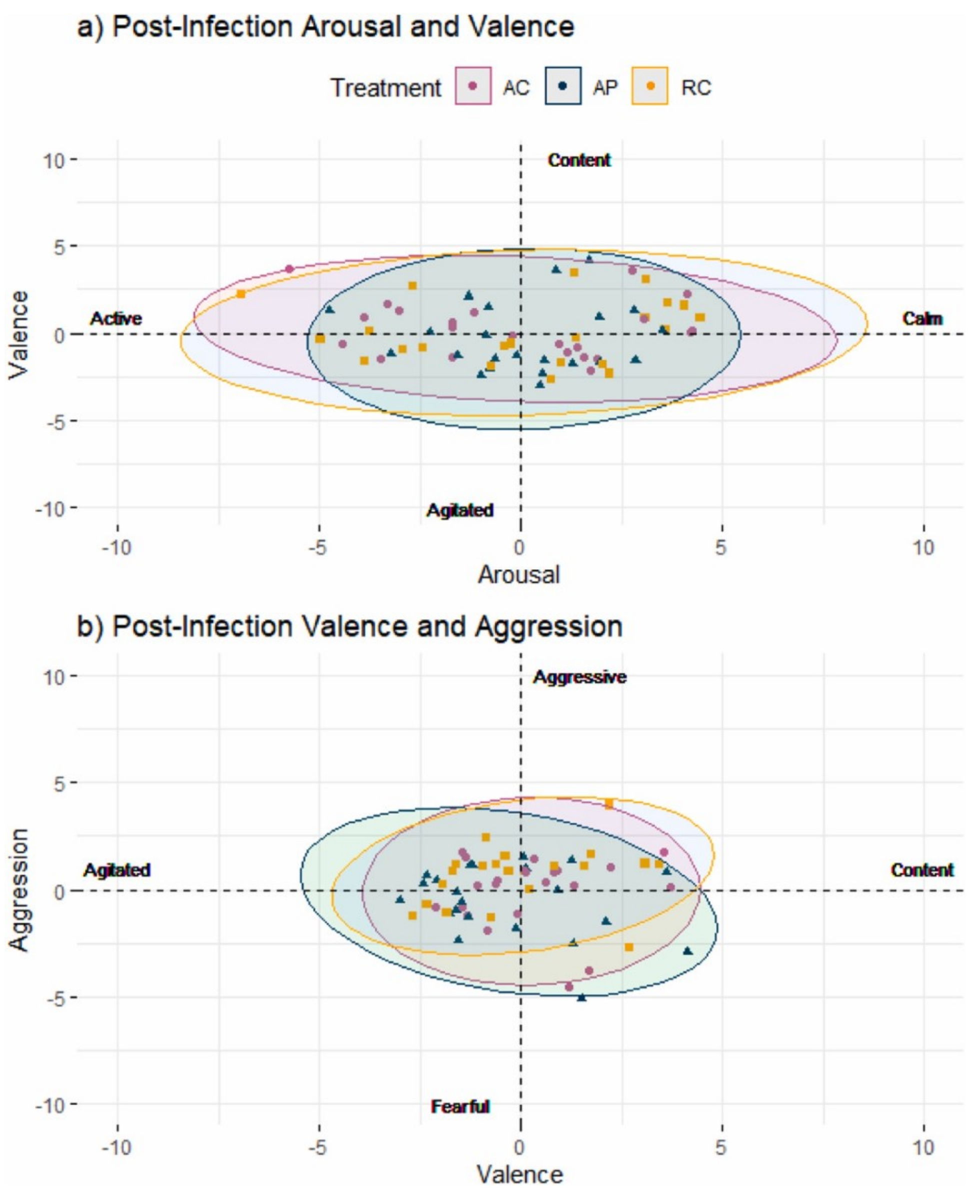

Fig. 4. Plots of pens over the infection period with a) PC1 (Arousal) on the x axis and PC2 (Valence) on the y axis and b) PC2 (Valence) on the x axis and PC3 (Aggression) on the y axis. Terms at both ends of the axes are anchors for the principal components. AC=ad-lib fed control, AP=parasitised and RC=restricted-fed control lambs.

4. Discussion

This study aimed to identify early indicators of GI parasitism and to understand its welfare impact on lambs. Subclinically parasitised lambs were more likely to stand and less likely to display eating behaviour than unparasitised lambs. QBA found that they scored lower on the dimension describing aggressivity than non-parasitised lambs.

T.circumcincta egg counts were low, as the study period extended 23 days after infection, capturing the prepatent phase of infection and beginning of the patent phase. Egg shedding begins in the patent phase, 15–21 days after infection (Roeber et al., 2013; Wood et al., 1995). As this study intended to identify early indicators of parasitism, this focus on early infection was justified. However, further longitudinal research on the patent phase is necessary to complete the understanding of behaviour changes throughout infection. Lambs infected with *T. circumcincta* in a previous study had a lower motion index, step count and fewer lying bouts than control lambs in the prepatent phase (Morris et al., 2022). This finding is similar to the present study's behavioural findings, despite the fact that Morris et al. (2022) studied lambs outdoors on pasture and used accelerometers to collect behavioural data.

AP lambs had a smaller increase in feed intake over time than AC lambs. The RC lambs had a lower mean intake than AP lambs, especially pre-infection and in the first 5 days of infection. The reason behind this lower intake is unknown. The purpose of the RC group was to separate behavioural and welfare impacts of hunger from those of parasite infection. This separation was rendered impossible by the RC lambs seemingly eating to satiation despite their restriction. The change in feed intake over time in AP lambs was significantly different from the pattern in AC lambs, and likely reflects the onset of parasite-induced anorexia. This reduction in feed intake has been reported in modelled subclinical *T.circumcincta* infection of lambs of the same age as the ones studied here (Laurenson et al., 2011). Some differences in lying, standing and eating behaviour across the three scan samples likely reflect the lambs' daily routine; they were fed between 9:00 h and 11:00 h every morning, and scans 1, 2 and 3 occurred at 13:00 h, 13:30 h and 14:00 h, respectively. Scan 1 was closest to feed time and more pellets likely remained in feed boxes than during later scans. The decreased likelihood of lying during scan 1 may reflect the increased likelihood that lambs were standing and eating.

Behaviour categories were mutually exclusive in this study's ethogram, therefore standing can be considered inactive behaviour. These results reflect previous findings where activity in many species was reduced during a health challenge (Gauly et al., 2007; Ghai et al., 2015; Hart, 1988; Morris et al., 2022). Although the exact reason for increased standing of AP lambs cannot be confirmed, it is possibly due to abdominal discomfort caused by abomasal damage inflicted by parasite larvae. *T.circumcincta* larval stages cause most pathogenic effects, as opposed to its adult stages (Roeber et al., 2013). Larvae development creates nodules in the abomasal mucosa and causes considerable damage to parietal cells, which increases the abomasum pH (Anderson et al., 1985; McKellar, 1993). Standing immobile has been reported as a reaction to castration pain in lambs since it avoids or reduces stimulation of the hyperalgesic tissue (Molony et al., 1993; Molony and Kent, 1997). It is possible that parasitised lambs were more likely to stand immobile to avoid stimulating their damaged abomasal tissue. This result leads us to accept our hypothesis that parasitised lambs reduced their activity levels compared to uninfected lambs.

Probably due to parasite-induced anorexia, the likelihood of observing eating behaviour remained low after scan 1 for AP lambs, whereas control lambs were just as likely to be eating during other scans. This reflects previous studies' findings where reduced feeding bouts in parasitised ruminants in varied experimental environments with varying levels and types of infection were reported (Fox et al., 2013; Hutchings et al., 2000b, 2002). Sheep have the ability to make complex grazing decisions to reduce parasite ingestion on pasture (Bricarello et al., 2023; Hutchings et al., 1999) but the experimental environment of this study did not allow for changes in feeding strategy. Based on the results, we accepted the hypothesis that parasitised lambs reduced their feeding activity.

Reduced play and socialising are components of sickness behaviour in many mammalian species (Dantzer and Kelley, 2007; Hart and Hart, 2019; Johnson, 2002; Weary et al., 2009). One study reported that when parasitised with *T.circumcincta* on pasture, social contacts between parasitised lambs were reduced compared to those between non-infected lambs (Morris, 2022). Contrary to these previous findings, the reduction in social behaviour after infection was seen across all treatment groups in this study. We rejected our hypothesis that parasitised lambs would reduce their social behaviour. Interactions between lambs could have decreased over time as the lambs aged and became accustomed to their surroundings. Social interactions are subject to breed differences, with English lowland breeds and Scottish hill breeds such as the ones in this trial being some of the least gregarious in outdoor settings (Dwyer and Lawrence, 1999). Further research in different breeds with focal observations of young lambs could shed more light on the dynamics of play and social behaviour during parasite infection. Play is influenced by the environment (Berger, 1979) but the pens used here were relatively bare, so space for play and social interaction may have acted as a limiting factor (Berger, 1979). That RC lambs′ play bout duration increased over time post-infection could be because they were a particularly playful or aggressive group of lambs, as shown through their non-significantly higher aggression loadings in QBA pre-infection. It was not possible to differentiate between antagonistic and playful bouts of head-butting and jumping during observations, so it is unclear if the RC lambs were truly more aggressive, or if they were simply more playful.

The PCA′s PC3 described a spectrum of behaviour from freezing alert to antagonistic social interactions. Post-infection, AP lambs′ behaviour was characterised by this alert freezing response, differing from RC lambs who had higher loadings on the aggression side of the axis. This reflects non-significant results in the pre-infection period where RC lambs had higher aggression loadings than AP lambs. It is possible that sick prey animals would increase their vigilance behaviour, as they are more vulnerable to predators. Lambs experiencing pain showed more vigilant behaviour in the presence of predators (Young, 2006). On pasture, observers scored inappetent sheep as more `reluctant`, `tense` and `wary` than control sheep, although the reason for their inappetence was not reported (Grant et al., 2018). These findings suggest that qualitative assessments of behavioural expression could contribute to identifying GI parasitism in sheep. This leads us to accept the hypothesis that parasitised lambs experienced a negative mental state.

The gut fill score may have been too crude to account for minor differences between lambs, and could only detect significant welfare impacts. This score had been useful as part of a wider welfare assessment index due to its good inter-observer agreement (Phythian et al., 2013). Rumen fill is often used in cattle studies but rarely appears in sheep trials (Zufferey et al., 2021). Its use did not lead to any analysis or conclusions in this study, therefore we must reject the hypothesis that parasitism causes lower gut fill scores. In this experiment, FSS was not associated with FEC. In one study, FSS had a low positive phenotypic correlation with FEC, although the FSS scale used was not described in detail (Bisset et al., 1992). Contrarily, Morris et al. (2000), (2005) found an increased FSS in their low FEC line of Romney sheep. Other studies found low genetic correlations between FEC and FSS in Merino sheep. FSS was an indicator of scouring, but it was different from FEC as an indicator of infection (Pollott et al., 2004). This reflects our FSS findings, leading us to reject our hypothesis that parasitised lambs would have higher FSS.

The GLMMs used to analyse behavioural data met the assumption of linear residuals, but the dispersion of the residuals was not entirely homogenous. This is likely due to sources of variation that were unaccounted for during data collection. This limitation was considered when interpreting the results of the models. Further work using models that account for nonlinear patterns of behaviour over time could help address this.

These findings could be applied to on-farm monitoring early behavioural indicators of parasitism, such as lambs standing immobile. Digital technologies like accelerometers could monitor this type of behavioural change remotely, while video cameras and machine-learning algorithms have the potential to detect immobile lambs in a barn. These tools could support farmers in early identification of infected animals and encourage prompt, individual treatment. The finding that parasitism may lead to negative mental states through increased fear is important if lamb welfare is to be improved. As parasitism is ubiquitous in grazing sheep, the implications of poor welfare in infected animals are wide-reaching.

5. Conclusion

Early indicators of disease are crucial to encouraging prompt treatment of health issues in extensively farmed sheep and lessening their impact on animal welfare. We demonstrate that subclinically parasitised lambs increased standing behaviour and decreased eating behaviour over time compared to non-parasitised lambs. These changes have the potential to act as early indicators of GI parasite infection. If behaviour can be monitored remotely by digital technology in extensively farmed sheep, infection could be detected early and at the individual level without gathering the flock. The QBA results suggest that parasitised lambs experienced more negative affective states linked to fear and anxiety compared to non-parasitised lambs. This finding contributes to the small body of evidence that GI parasitism, even at a subclinical level, negatively impacts lamb welfare not only in the health domain but in the behaviour and mental domains as well. Future research into tools to monitor early behavioural indicators such as accelerometers could help improve lamb welfare and encourage prompt and individual treatment, which could contribute to fighting anthelmintic resistant. Repeating similar studies in extensive conditions and with different sheep breeds could help apply the findings to the variety of commercial sheep farming conditions.

CRediT authorship contribution statement

Fiona Kenyon: Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing. **Jessica E Martin:** Data curation, Formal analysis, Methodology, Supervision, Writing – review & editing. **Emma M Baxter:** Conceptualization, Methodology, Visualization, Writing – review & editing. **Cathy M Dwyer:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing. **Michelle C Reeves:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization,

Writing – original draft, Writing – review & editing. **Naomi Booth:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. **Naomi J Fox:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing. **Jo Donbavand:** Investigation, Methodology, Writing – review & editing. **Mhairi Jack:** Conceptualization, Investigation, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2024.106323.

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